THE UNIVERSITY OF ALBERTA

DETECTION OF SOME CHEMICAL CHANGES CAUSED BY PSYCHROPHILIC MICRO-ORGANISMS IN MILK

A THESIS

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SUMMARY

An attempt has been made to relate the number of psychrophiles in milk to a product of metabolism. Peptides were chosen as an intermediate by-product occurring early in the decomposition of protein.

Fifteen strains of psychrophiles were isolated from milk and milk products and identified into genus and species. In the course of identifying the strains of psychrophiles the various cultural and biochemical reactions were studied at 37°, 25°, 15° and 4°C. The ability of all strains of psychrophiles to ferment carbohydrates was strikingly different at 15° and 4°C. The non-psychrophilic pseudomonads, subjected to the same experimental conditions, gave no different result in their behavior on carbohydrates at 15° and 4°C.

The ability of psychrophiles to liquefy gelatin and hydrolyse starch has also been found to be markedly different at different temperatures, especially at 15° and 4°C, suggesting that this group of micro-organisms may show quite different biochemical behavior at these two temperatures. The possible mechanisms responsible for a difference in biochemical behavior at different temperatures are discussed. Disregarding the differences in reaction caused by different incubation temperatures, the most commonly occurring genera, in the psychrophiles isolated were Pseudomonas and Alcaligenes.

To determine the relationship between numbers of psychrophiles in milk and the amount of peptide liberated, the psychrophiles were inoculated separately into sterilized milk and the liberated peptide was separated by dialysis and identified by paper chromatography with

three different solvent systems. The amount of peptide separated from each sample was measured spectrophotometrically at 580 mu in ninhydrin solution against a known molar concentration of a free amino group as control. The psychrophiles liberated a peptide from milk containing arginine and glycine, and occasionally an amino acid, histidine.

The same comparison was made for bacteria occurring naturally in raw milk. The same peptide was liberated, and with a further increase in the number of cells an amino acid, histidine, was also liberated. Almost all the strains isolated from milk and milk products showed a similar relationship between the number of cells and the amount of peptide liberated which increased up to a point and suddenly decreased. Different genera and different species within the genus produced different breaking points. The results suggest the possibility that psychrophiles might re-arrange the amino acid sequence before they liberate peptide. However, because of the variable amount of peptide liberated by different strains of psychrophiles, this intermediate compound cannot be recommended as a reliable measure of the number of psychrophiles in milk.

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INTRODUCTION

Continued improvement in the keeping quality of milk and milk products has always been the endeavour of the dairy industry. Storage at low temperatures has been one of the customary means of preserving such products but this in turn has led to difficulties with psychrophilic bacteria.

Many definitions have been proposed for psychrophilic bacteria. For example, Thomas *et al*. (1959) defined psychrophiles as bacteria which were able to grow at or below 5°C and did not survive pasteurization. Van der Zant & Moore (1955) considered that all psychrophiles would grow at a temperature of 20°C or above. Foster *et al*. (1957) defined psychrophiles as those which are able to grow below 15°C whereas Ingraham & Stokes (1959) suggested as a criterion those bacteria which form easily visible colonies on solid media within 14 days at 0°C. The American Public Health Association (1960) recommended a temperature of 5 to 7°C for seven to ten day incubation for enumeration of psychrophiles. The most recent reviews concerning the definition of psychrophiles have been written by Jezeski (1954), Thomas *et al*. (1960) and Witter (1961).

As a consequence of the ambiguity of the definition of psychrophiles, confusion has existed in the identification and classification of such bacteria. However, the general opinion now is that the most commonly encountered psychrophiles belong to the genus *Pseudomonas*, this seems to be true not only for dairy products,

but also for meat, fish, poultry and eggs.

Ayres (1960) has advocated the need for a systematic study on the identification of species within the genus, and recommended that the reaction rate and profuseness of growth at each of several temperatures, such as, 5°, 15°, 25°, 37° and 42°C, should be determined.

Criticisms of Bergey's Manual of Determinative Bacteriology (Breed et al., 1957) have been made by De Ley (1960) and Lysenko (1961) on the grounds that from the time of Migula the definition of genus has been based mainly upon morphology and not upon biochemical and cultural characteristics, such as the distinction between oxidative and fermentative utilization of glucose based on the iodoacetate test. While this is cogent, neverthless, the term 'oxidative or fermentative metabolism' should be used carefully, as little is known about the other pathways by which glucose may be utilized.

The absence of the Embden-Meyerhof system of glucose fermentation in different pseudomonads was demonstrated by Norris & Campbell (1949), and a relatively new pathway in which 2-keto-gluconate is an intermediate was proposed by Entner & Doudoroff (1952); this has since been confirmed by several other investigators.

Many methods have been advocated for the enumeration of psychrophiles in milk but none have been completely satisfactory. In "Standard Methods for the Examination of Dairy Products" (The American Public Health Association, 1960), the method advocated for the enumeration of psychrophiles has the disadvantage of a long

incubation period. Czenge & Doan (1958) measured the keeping quality of cream, condensed milk and milk by the tetrazolium reduction method and they claimed that this method showed promise for the examination of raw milk held at 3 - 5°C. Unfortunately the accuracy and reproducibility of the test is not sufficient for it to be generally adopted.

Thomas et al. (1959) suggested using a half-hour methylene blue and a half-hour resazurin test at 37°C together with the clot-on-boiling test for refrigerated milk after pre-incubation for 24 hours at 22°C in an attempt to develop a simple routine test. The results, however, showed that the sensitivity of these tests was not sufficient for refrigerated milk.

Neither the methylene blue (Johns, 1952; Harris et al., 1956) or resazurin tests (Johns, 1952; Broitman et al., 1958; Greene & Jamison, 1959) have proved good indices for keeping quality of raw or pasteurized milk held at refrigeration temperature.

Trials of the clot-on-boiling test have been reviewed by Thomas et al. (1959) who showed that several days before a positive clot-on-boiling end-point, samples of refrigerated milk developed a distinct fruity and/or rancid aroma. Titratable acidity and pH of milk during refrigeration storage showed no appreciable change as a result of psychrophilic activity (Day & Doan, 1956; Thomas et al., 1959). The development of off-flavor and odors have been noted (Peterson & Gunderson, 1960) in advance of the development of large number of psychrophiles.

Witter & Tuckey (1960) have studied the generation time of some psychrophilic pseudomonads in nutrient broth and in skim milk, and a sudden shortening of the generation time has been observed after an incubation period (approximately 20 days at 4°C). This may be interpreted as a result of an accumulation of intermediates which could have stimulated enzymes in template form, or caused a spacial re-arrangement of enzymes and/or substrate to stimulate inactivated enzyme. If such intermediates could be detected, it should be possible to predict the activity of psychrophilic bacteria, and also the keeping quality of milk and milk products held at refrigeration temperatures.

The unique ability of heterotrophic micro-organisms to degrade carbohydrates rapidly for the production of energy and intermediates for biosynthesis has caused much speculation with regard to the nature of the metabolic pathways. However, with the elucidation of the glycolytic mechanism by the Embden-Meyerhof reaction, the concept was evolved that this glycolytic pattern is the basic mechanism in all bacteria capable of carbohydrate utilization. Several observations (Stern et al., 1960; Gibbs, 1954; Weimberg, 1959; Weimberg, 1961) particularly with the Pseudomonas and Acetobacter species, have suggested that carbohydrate oxidation involved a completely different reaction to that proposed in the Embden-Meyerhof reaction, since the intermediates were formed in the presence of such enzyme inhibitors as fluoride and iodoacetate. Much attention has been paid to glucose oxidation without initial phosphorylation, since

Entner & Doudoroff (1952) postulated a pathway with Pseudomonas strains and identified the intermediates in the reaction.

Of 136 cultures of psychrophilic organisms isolated from milk (Bly & Eleaner, 1954) 87.5% were species of Pseudomonas. Schultze & Olson (1957) and Thomas et al. (1960) have listed the distribution of psychrophilic organisms in milk and milk products. The bacterial genera most commonly found to contain psychrophilic species are, Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Escherichia and Aerobacter. Though Witter (1961) has listed 21 genera which contain psychrophilic bacteria, the distribution demonstrated by Bly & Eleaner (1954), Schultze & Olson (1957) and Thomas et al. (1960) suggests that the main chemical changes brought about by the psychrophilic bacteria in milk and milk products are caused by Pseudomonas species. Since all Pseudomonas species can carry two or three different carbohydrate oxidation pathways, viz: Embden-Meyerhof, pentose-phosphate shunt, Entner-Doudoroff, the detection of such chemical changes may not be easy with the exception of residual lactose, and under such circumstances the accumulation of carbohydrate intermediates which stimulate cell-synthesis would be unlikely to occur.

Despite the well recognized importance of lipolytic bacteria in milk and milk products, little work has been done on lipolysis by psychrophilic bacteria except to demonstrate their presence or to show that a particular bacterium produced rancidity. The ability of psychrophiles to produce lipase (Nashif, 1953; Nashif & Nelson, 1953a, b, c, d) varies considerably between genera, between species

of the same genus, and even between strains of the same species. Olson et al. (1955) emphasized the fact that the type of contaminating organisms is of far greater importance in bringing about lipolysis than the number of cells. Thus although rancidity is one of the strongest off-flavors produced by psychrophiles, the detection of lipolytic intermediates does not seem to be a good method for estimating the keeping quality of milk because of the complexity of the end-products.

Psychrophiles with proteolytic activity have frequently been encountered, and the majority of psychrophiles isolated by Stark & Scheib (1936) and Thomas & Thomas (1947) were observed to be proteolytic. It was observed very early that a common failure of milk held at a low temperature was brought about by a proteolytic change (Pennington et al., 1913 - 14). The holding of milk at low temperature is selective against those bacteria which normally produce acid from lactose, and selective for the psychrophilic bacteria which preferentially attack fats and proteins. Although the liberation of active proteolytic enzymes produced by psychrophilic bacteria occurs at an optimum temperature (Peterson & Gunderson, 1960), neverthless heterotrophic bacteria require protein for their cell synthesis and therefore will attack it at any temperature.

After a series of degradation experiments, Hagihara (1960) postulated that protease from pseudomonads more closely resembles papain than trypsin or pepsin. Thus it is possible that if the majority of psychrophilic bacteria in milk and milk products belong to the genus Pseudomonas, and if these organisms carry a papain-

type protease, that most psychrophiles present in milk might liberate relatively short chain peptides from milk proteins, as proposed by Sanger & Tuppy (1951), Waley & Watson (1954) and Johnson & Herriott (1956), and such liberated peptides would be utilized for cell synthesis after being hydrolyzed into amino acid moieties.

Numerous instances are now known in which peptides supply a limiting amount of an essential amino acid to autotrophic micro-organisms more efficiently than an equimolar amount of the free amino acid (Kihara & Snell, 1952; Virtanem & Nurmikko, 1951; Meinhart & Simmonds, 1955). Kihara, Ikawa & Snell (1961) have emphasized that such efficient utilization does not imply utilization of the peptides without intracellular hydrolysis. Under their experimental conditions, Streptocuccus faecalis hydrolyzed such peptides rapidly. In many instances the results were best explained by assuming the existence of an independent mechanism for the active uptake of the peptides followed by its hydrolysis. De Koning (1960) isolated peptides from cheese and claimed that milk proteins should be cleaved into peptides before they could be further broken down to the amino acid level.

Since the generation rate of psychrophilic bacteria is relatively low in the temperature range in which milk and milk products are usually stored, there should be an appreciable time lapse between the liberation of peptides and their hydrolysis into amino acid moieties. Accordingly it appeared possible that the detection of peptides liberated by psychrophilic bacteria might be the basis for

a new method for estimating the number of psychrophilic cells in milk and hence its keeping quality.

EXPERIMENTAL PROCEDURES

1. Isolation of Psychrophilic Micro-organisms

Samples were obtained of high bacterial count raw milk, pasteurized milk, whipping cream, chocolate milk, cottage cheese and salad cream. After collection the samples were kept at 4°C for one week to allow further growth of the psychrophilic bacteria. Every sample was plated on plate count agar (American Public Health Association, 1960) and incubated at 4°C for 7 days. Plates with colony counts between 10 and 50 were selected and each colony was streaked on fresh plate count agar. The plates were incubated at 4°C for 5 days, and colonies thus obtained were streaked again on plate count agar. After 5 day's incubation at 4°C, each colony was examined microscopically for morphology and Gram-staining. The strains were kept on tryptone-dextrose-yeast-extract-agar slants (American Public Health Association, 1960). Fifteen strains were selected, based on rapidity of growth at low temperature, and were identified into genus and species.

2. Identification of Isolated Psychrophiles

Except for morphology and Gram-staining observations, the cells used for identification were harvested as follows.

A loopful of the stock culyure from a slant was transferred into nutrient broth and incubated at 4°C for 7 days, centrifuged at 1,000 x g for 15 min., washed once with sterilized and chilled 0.9% NaCl solution, centrifuged once at 1,000 x g for 15 min., and

re-suspended into 0.9% NaCl solution. The cell concentration was adjusted to 0.50 optical density by a Bausch and Lomb 340 model spectrophotometer at 490 mμ in 0.9% NaCl solution. One tenth ml of such suspension was inoculated into 5 ml of medium. All the reagents used were C.P. grade and media unless specified otherwise were Difco dehydrated products. Sterilization was, unless otherwise stated, done in the autoclave at 121°C for 15 min.

a) Carbohydrate utilization

A basal medium containing 0.5% yeast extract, 0.3% beef extract, 0.5% peptone (all w/v), 10 ml/1,000 ml each of Salts A and B (see below) was distributed in 4 ml amounts into test tubes, which contain Durham's tubes, and was then sterilized. One ml of 5% (w/v) carbohydrate, after sterilization and chilling, was added aseptically to the basal medium. Salt A solution consisted of KH_2PO_4, 25g; K_2HPO_4, 25g; distilled water, 250 ml. Salt B solution contained $MgSO_4 \cdot 7H_2O$, 10g; $FeSO_4 \cdot 7H_2O$, 0.5g; NaCl, 0.5g; $MnSO_4$, 0.5g; distilled water, 250 ml.

The following carbohydrates were tested for growth, gas production, acid or alkali formation by titration with 0.1 N NaOH: glucose, galactose, fructose, mannitol, rhamnose, arabinose, xylose, sorbitol, lactose, maltose, sucrose, raffinose, inulin, starch, neutralized lactic acid (with NaOH) and glycerol.

b) Starch hydrolysis

The medium used contained 1% soluble starch, 0.3% beef extract, 0.5% yeast extract and 2% agar (all w/v). Plates were prepared from this medium and the strains streaked on the surface and incubated. Hydrolysis of starch was detected by spraying iodine solution on the surface of the plate: those plates which exhibited a clear zone around the streak being recorded as positive.

c) Indole formation

Bacto-peptone medium was used and indole was detected by the addition of 2 ml of reagent 1, followed by 1 ml of reagent 2 into 5 ml of medium. Reagent 1 was composed of p-dimethylamidobenzaldehyde, 4g; absolute ethyl alcohol, 380 ml; concentrated HCl, 80 ml. Reagent 2 consisted of saturated aqueous solution of $K_2S_2O_8$ prepared by saturating 100 ml of cold distilled water with 2g of the salt. The red color developing in 5 min. was recorded as positive for the presence of indole.

d) Nitrate reduction

To 5 ml medium of 0.3% beef extract, 0.5% peptone, 0.1% KNO_3 (prepared in this laboratory), 1 ml each of α-naphthylamine and sulphanilic acid reagents were added to detect nitrite, after incubation. Durham's tubes were inserted to detect nitrogen gas liberation. Production of red color after 30 min at room temperature was recorded as positive.

The α-naphthylamine reagent was prepared by dissolving 1.0g of α-naphthylamine in 20 ml of distilled water, and filtering through a Whatman #1 filter paper; to the filtrate, 180 ml acetic acid

(sp. gr. 1.04) was added. For the other reagent 0.5g of sulphanilic acid was dissolved in 15 ml glacial acetic acid (sp. gr. 1.04).

e) Litmus milk

Bacto-dehydrated litmus milk (105g) was suspended in 1,000 ml of distilled water and blended in a Waring Blendor for 5 min. The following changes were recorded: Acidic or alkaline reaction, reduction, coagulation and peptonization.

f) Gelatin liquefaction

The medium used was specially prepared and consisted of beef extract, 0.3%; peptone, 0.5%; gelatin 12.0% (all w/v). After an appropriate time for incubation at the desired temperature the inoculated tubes were immersed into ice-water where necesarry and the tubes which did not solidify were recorded as positive.

g) Pigment formation

Pigment formation was determined on Bacto-potato-dextrose agar (non-acidified) with a final pH of 6.5.

All tests were made in duplicate at 4°, 15° 25° and 37°C together with controls at each temperature. Care was taken to obtain maximum randomization on each test. The growth of strains was measured by turbidity, and recorded in 10 classes. The development of gas was measured by volume in Durham's tubes and recorded in 5 classes. The acid or alkali production from the carbohydrate was measured by titrating with 0.1 N NaOH to the phenolphthalein end-point, and the value obtained corrected by substracting the value of the

uninoculated control. Titration values less than that of the control were classified as alkali production, and a chi square value less than the 5% point was classified as non-acid production.

3. Enumeration of Bacteria

The number of bacterial cells was determined on plate count agar incubated at 4°C for 10 days.

4. Isolation of Peptides

Peptides were obtained by dialyzing 100 ml of milk sample against 1,500 ml of distilled water for 48 hours. (Trial experiments had shown that 24 hours was insufficient for dialysis with the membrane used which was from Fisher Scientific Company, catalogue # 8-667, wall-thickness of the membrane, 0.00072 inch).

The dialyzed solution thus obtained was condensed in vacuum at 50°C in a water bath. To eliminate the lactose effect for paper chromatography, a sufficient amount of sodium periodate (6g/1,500 ml dialysate) was added during the condensation. Iodates reacted with threonine, serine and hydroxylysine, but no reaction took place with peptides containing such amino acids. The excess IO_4, as well as IO_3, was separated from the peptide solution by filtering through a Whatman #1 filter paper, after the sample was placed at 4°C for 3 hours. The final volume was adjusted to 5 ml with distilled water. Isolation of peptides by trichloroacetic acid solution or by ethyl

alcohol was not as successful as by dialysis.

5. Separation of Peptides

Paper chromatography was applied to separate the peptides. To select suitable solvent systems the following experiment was made. Isoelectric casein, prepared by precipitating reconstituted skim milk powder with 0.1 N HCl, was washed once with distilled water and dissolved and adjusted to pH 9.6 with 0.1 N NaOH. Again the casein solution was precipitated with 0.1 N HCl at pH 4.7 and washed with distilled water once, and dissolved with 0.1 N NaOH. The final pH was adjusted to 7.1. The recovery of the final casein was about 25%. The casein concentration was adjusted to approximately 5% with distilled water and 100 mg peptidase (source - hog intestine, Nutritional Biochemical Corp.) was added per 100 ml of casein solution and the mixture incubated at 37°C for 60 min. Peptides thus obtained were spotted on Whatman #1 filter paper and suitable solvent systems were selected. Several solvent systems were tested by three chemically pure peptides (glycyl-DL-valine, DL-alanyl-DL-phenylalanine, DL-leucyl-DL-leucine, all obtained from Nutritional Biochemical Corp.) and by the peptides which were hydrolyzed from casein by peptidase. As a result three systems were selected as follows:

1) n-butanol - methylethylketone - 25% NH_4OH - water
   (5 : 3 : 1 : 1) (De Koning, 1960)
2) n-butanol - ethyl alcohol - water
   (10 : 10 : 5) (Hardy, Holland & Mayler, 1955)

3) n-butanol - glacial acetic acid - water

(4 : 1 : 5) (Levy & Chung, 1953)

6. Detection of Peptides

Two identical sheets of chromatograms were prepared by dissolving 1-2 mg C.P. peptide in 1 ml of distilled water and 10 µl was spotted on each sheet. After an appropriate period for the development of the chromatograms (16 hours at room temperature for 35 cm) one paper was sprayed with 0.1% ninhydrin solution in 95% EtOH, and the other, with Biuret solution. The spots were developed by drying the papers at room temperature under a hood. Ninhydrin showed much stronger colored spots than the Biuret solution which were very faint. Even with milk casein (protein concentration 2 mg/ml), 10 µl did not produce a strongly coloured spot with Biuret solution. Thus ninhydrin was used to detect peptides.

7. Determination of Amino Acid Composition of a Peptide

The peptide separated by paper chromatography was easily eluted by a small amount of distilled water by a capillary technique. A recovery of 60 - 70% was obtained, calculated by comparison of the color produced from a solution of known molarity with ninhydrin, and measured by optical density at 580 mµ.

The eluted peptide, condensed to 0.5 ml or less by vacuum in a 50°C water bath, was pipetted into a lyophilizing bacteriological tube, and 1 ml of 6 N HCl was added. The tube was evacuated to 9.0 - 9.5 cm Hg, sealed, and hydrolyzed at 121°C for 8 hours. After

hydrolysis, the tube was carefully broken and excess HCl was evacuated by the water pump at 50°C in a water bath.

Two-dimensional paper chromatography with two different solvent systems, (modified from Wolfe, 1957) were used as follows: (a) n-butanol - glacial acetic acid - water and n-butanol - EtOH - water and (b) water saturated phenol and n-butanol - glacial acetic acid - water.

One mg each of 19 commercially available natural L-amino acids (Nutritional Biochemical Corp.) was dissolved in 1 ml of 0.1 N HCl, and 10 ul was spotted on the paper. The amino acid was dissolved in 0.1 N HCl in order that there should always be a trace of HCl present because on hydrolysis of a peptide with 6 N HCl and evacuation, a small amount of HCl occasionally might be left and might affect the affinity of the hydrolyzed amino acid with solvent systems thus giving variable results.

8. Peptide Determination

After the chromatographic paper was developed by spraying a small amount of 0.1% ninhydrin solution in ethanol, the spot was eluted with 1 ml of distilled water by a modified technique developed by Tuckey et al. (1959). The solution thus obtained was diluted with 3 ml of 0.4% ninhydrin solution in ethanol and the concentration of the peptide was measured by a Bausch and Lomb 340 model spectrophotometer at 580 mμ, in comparison with a known molar amino acid

as a standard developed on the same chromatographic paper.

RESULTS

1. Identification of Psychrophilic Strains

The fermentative ability of fifteen strains of psychrophiles on sixteen carbohydrates at different incubation temperatures is shown in Tables 1 - 15. There are marked differences in behavior in gas production and acid values with different incubation temperatures, especially between 15° and 4°C, even when it was known that almost the same cell concentrations were present at the completion of incubation. Trial experiments, and prolonged incubation for 7 and 15 days at 15° and 4°C, respectively, did not change the overall results with gas production. Likewise diminishing or increasing the inoculum size (0.2 and 0.65 O.D. at 490 mu, respectively) effected no change.

To determine whether the difference in gas production was caused by either the inoculation procedures or incubation temperatures, fifteen relatively fast growing strains of non-psychrophilic pseudomonads (5 strains of Ps. fluorescens, 5 of Ps. aeruginosa, 3 of Ps. fragi, 2 of Ps. putrefaciens), were inoculated into six representative carbohydrate media, using the same inoculation procedures as hitherto. These strains were kindly supplied by the National Research Council (Division of Applied Biology), Prairie Regional Laboratory (Saskatoon), and the University of British Columbia (Dept. of Dairying). Though the generation time of such strains was not as short as the psychrophilic pseudomonads isolated, the

overall results of the carbohydrate fermentation did not produce any difference between 15° and 4°C as was observed with the psychrophiles.

Table 1 The fermentative ability of strain #1 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol		
		growth*1	gas *2	tit value*3	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.05	-		0.05	-		-			-			0.1	-		-			-			0.05	-	
		0.05	-		0.05	-		-			-			0.1	-		-			-			-		
	48	0.1	-	.010	0.1	-	.020	-			-			0.1	-	alk	-			-			0.1	-	alk
		0.1	-	.020	0.1	-	.015	0.1	-	.030	-			0.1	-	alk	-			-			0.05	-	alk
25°	24	0.5	-		5	-		0.2	-		0.1	-		20+	-		0.2	-		20+	-		20++	-	
		0.5	-		5	-		0.2	-		0.2	-		20+	-		0.2	-		20+	-		20++	-	
	48	5	-	.060	20+	-	.085	20+	+	.080	20+	+	.060	20+	-	alk	20+	+	.045	20+	-	.060	20++	-	alk
		5	-	.105	20+	-	.085	20+	+	.090	20+	+	.065	20+	-	alk	20+	+	.050	20+	-	.050	20++	-	alk
15°	24	0.5	-		5	-		0.05	-		0.1	-		5	-		0.1	-		5	-		20++	-	
		0.5	-		5	-		0.05	-		0.1	-		5	-		0.1	-		5	-		20++	-	
	48	5	-		20+	-		0.5	-		0.2	-		20++	-		5	-		20++	-		20++	-	
		5	-		20+	-		0.5	-		0.2	-		20++	-		5	-		20++	-		20++	-	
	72	9	-	.100	20++	-	.080	20+	+	.070	9	+	.050	20++	-	alk	20++	-	.030	20++	-	NA	20++	-	NA
		9	-	.105	20++	-	.090	20+	+	.070	9	+	.040	20++	-	alk	20++	-	.025	20++	-	NA	20++	-	NA
4°	144	0.2	-		0.2	-		9	-		5	-		9	-		5	-		9	-		9	-	
		0.2	-		0.2	-		9	-		5	-		9	-		5	-		9	-		9	-	
	192	5	-		9	-		20+	-		20+	-		9	-		20+	-		9	-		20+	-	
		5	-		9	-		20+	-		20+	-		9	-		20+	-		9	-		20+	-	
	240	20++	-	.060	20++	-	.040	20++	-	NA	20+	-	alk	20++	-	alk	20++	-	NA	20++	-	NA	20++	-	NA
		20++	-	.050	20++		030	20++	-	NA	20+	-	alk	20++	-	alk	20++	-	NA	20++	-	NA	20++	-	NA

incubation temp.	incubation hours	lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	-		?			0.2	-		-			0.1	-		?			0.2	-		0.2	-	
		0.1	-		-			0.2	-		-			0.1	-		?			0.2	-		0.2	-	
	48	0.1	-	.055	-			0.5	+	.070	-			0.1	-	NA	?			0.2	+	NA*	0.5	+	.040
		0.1	-	.055	-			0.5	±	.070	-			0.1	-	NA	?			0.2	±	NA	0.5	±	.040
25°	24	20++	-		20++	-		5	-		9	-		9	-		9	-		0.5	-		5	-	
		20++	-		20++	-		5	-		9	-		9	-		9	-		0.5	-		5	-	
	48	20++	-	.110	20++	-	.130	20++	++	.090	9	-	.040	20++	-	NA	20++	-	.020	20+	-	alk*	20++	++	.030
		20++	-	.110	20++	-	.130	20++	++	.090	9	-	.045	20++	-	NA	20++	-	.020	20+	-	alk	20++	++	.030
15°	24	5	-		0.5	-		0.05	-		0.5	-		0.5	-		5	-		0.2	-		0.1	-	
		5	-		0.5	-		0.05	-		0.5	-		0.5	-		5	-		0.2	-		0.1	-	
	48	9	-		9	-		9	-		9	-		20++	-		9	-		20+	-		20+	-	
		9	-		9	-		9	-		9	-		20++	-		9	-		20+	-		20+	-	
	72	20++	-	.120	20+	-	.110	20++	-	.050	20++	-	.055	20++	-	NA	20+	-	.035	20++	-	alk	20++	-	.040
		20++	-	.105	20+	-	.110	20++	-	.050	20++	-	.050	20++	-	NA	20+	-	.035	20++	-	alk	20++	-	.035
4°	144	0.5	-		0.5	-		20+	-		0.2	-		0.5	-										
		0.5	-		0.5	-		20+	-		0.2	-		0.5	-		0.5	-		20+	-		5	-	
	192	5	-		5	-		20++	-		5	-		9	-		0.5	-		20+	-		5	-	
		5	-		5	-		20++	-		5	-		9	-		5	-		20++	-		20++	-	
	240	20++	-	.065	20++	-	.070	20++	-	.010	20+	-	.050	20+	-	alk	5	-		20++	-		20++	-	
		20++	-	.075	20++	-	.070	20++	-	.010	20+	-	.050	20+	-	alk	20+	-	.020	20++	-	alk	20++	-	NA
																	20+	-	.020	20++	-	alk	20++	-	NA

*1 growth: optical density (at 490 mu) x 10

*2 gas: gas production: -, negative; ±, very little; +, slight ++, good; +++, abundant

*3 tit value: titration value: actual titration minus control titration value (range of control .045 - .050)

The O.D. range for growth was categorized into 8 groups and the higher reading taken, e.g. any O.D. in the range 0.5 - 0.9 is reported as 9. 0.05 value represents the case where growth is recognized, yet no O.D. reading could be measured.

* NA - no acid production

* alk - alkali production, by phenolphthalein end-point

Table 2 The fermentative ability of strain #2 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.05	-		0.1	-		-			-			0.1	-		-			0.1	-		0.2	-		-			-			0.2	-		-			-			0.2	-		0.05	-		0.05	-	
		0.05	-		0.1	-		-			-			0.1	-		-			-			0.2	+		-			-			0.2	-		-			-			0.2	-		0.05	-		0.05	-	
	48	0.5	-	.045	0.2	-	.035	-			-			0.2	-	.045	-			0.2	++	.065	0.5	++	.045	-			-			0.5	-	.060	-			-			0.2	-	.020	0.1	-	NA	0.1	-	.030
		0.5	-	.050	0.2	-	.035	-			-			0.2	+	.045	-			-		.010	0.5	++	.064	-			-			0.5	-	.060	-			-			0.2	-	.020	0.1	-	NA	0.1	-	.030
25°	24	0.2	-		5	-		0.2	-		0.2	-		20++	+		0.2	-		20++	++		20++	++		9	-		9	-		0.5	-		5	-		9	-		9	-		0.05	-		0.2	-	
		0.2	-		5	-		0.2	-		0.2	-		20++	+		0.2	-		20++	++		20++	++		9	-		9	-		0.5	-		5	-		9	-		9	-		0.05	-		0.2	-	
	48	5	-	.070	20+	-	.060	20+	+	.090	20+	+	.070	20++	++	.125	20+	+	.055	20++	+++	.095	20++	+++	.080	9	-	alk	9	-	alk	9	-	.070	5	-	alk	20++	-	alk	20++	-	NA	0.1	-	alk	0.5	-	.040
		5	-	.065	20+	-	.040	20+	+	.100	20+	+	.065	20++	++	.120	20+	+	.055	20++	+++	.095	20++	+++	.080	9	-	alk	9	-	alk	9	-	.070	5	-	alk	20++	-	alk	20++	-	NA	0.1	-	alk	0.5	-	.035
15°	24	0.5	-		5	-		0.1	-		0.1	-		9	-		0.1	-		20+	-		20++	-		5	-		5	-		0.2	-		0.5	-		0.5	-		0.5	-		0.5	-		0.2	-	
		0.5	-		5	-		0.1	-		0.1	-		9	-		0.1	-		20+	-		20++	-		5	-		5	-		0.2	-		0.5	-		0.5	-		0.5	-		0.5	-		0.2	-	
	48	5	-		9	-		0.5	-		0.2	-		20+	++		0.5	-		20+	+		20++	+++		5	-		5	-		5	-		5	-		20++	-		20++	-		5	-		9	-	
		5	-		9	-		0.5	-		0.2	-		20+	++		0.5	-		20+	+		20++	+++		5	-		5	-		5	-		5	-		20++	-		20++	-		5	-		9	-	
	72	20+	+	.060	20++	-	.050	20++	+	.050	20+	±	.040	20++	++	.090	20+	±	.045	20++	++	.050	20++	+++	.080	20+	-	alk	9	-	alk	20+	-	.050	9	-	alk	20++	-	alk	20++	-	alk	20++	-	alk	20++	-	.030
		20+	+	.070	20++	-	.050	20++	+	.060	20+	±	.050	20++	++	.090	20+	-	.045	20++	++	.060	20++	+++	.080	20+	-	alk	9	-	alk	20+	-	.040	9	-	alk	20++	-	alk	20++	-	alk	20++	-	alk	20++	-	.030
	144	0.2	-		0.2	-		9	-		5	-		9	-		5	-		9	-		9	-		5	-		5	-		9	-		0.5	-		0.5	-		0.5	-		20+	-		20+	-	
		0.2	-		0.2	-		9	-		5	-		9	-		5	-		9	-		9	-		5	-		5	-		9	-		0.5	-		0.5	-		0.5	-		20+	-		20+	-	
4°	192	0.5	-		9	-		20++	-		20+	-		20+	-		20+	-		20++	-		20++	-		9	-		5	-		20++	-		5	-		9	-		9	-		20+	-		20++	-	
		0.5	-		9	-		20++	-		20+	-		20+	-		20+	-		20++	-		20++	-		9	-		5	-		20++	-		5	-		9	-		9	-		20+	-		20++	-	
	240	20++	-	.040	20++	-	.030	20++	-	.015	20++	-	NA	20++	±	.050	20++	-	.030	20++	±	.040	20++	++	.030	20++	-	alk	20++	-	alk	20++	-	NA	20+	-	alk	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA
		20++	-	.040	20++	-	.035	20++	-	.015	20++	-	NA	20++	±	.045	20++	-	.040	20++	±	.040	20++	++	.030	20++	-	alk	20++	-	alk	20++	-	NA	20+	-	alk	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 3 The fermentative ability of strain S6 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	±		0.1	-		-			-			0.5	+++		-			0.1	+		0.5	+		-			-			0.05	-		-			-			-			0.1	-		0.05	-	
		0.1	±		0.1	±		-			-			0.5	+++		-			0.1	+		0.5	+		-			-			0.05	-		-			-			-			0.1	-		0.05	-	
	48	0.5	+	.055	0.2	++	.055	-			-			0.5	+++	.085	-			0.5	++	.035	0.5	+++	.055	-			-			0.2	-	.030	-			-			-			0.2	-	NA	0.2	-	NA
		0.5	+	.065	0.5	++	.040	-			-			0.5	+++	.085	-			0.5	++	.035	0.5	+++	.050	-			-			0.2	-	.030	-			-			-			0.2	-	NA	0.2	-	NA
25°	24	0.5	+		0.5	+		0.5	-		0.5	-		20++	+		0.5	-		20++	+		20++	+++		20+	-		20++	-		20+	++		5	-		20+	-		9	-		20+	-		20+	-	
		0.5	+		0.5	+		0.5	-		0.5	-		20++	+		0.5	-		20++	+		20++	+++		20+	-		20++	-		20+	++		5	-		20+	-		9	-		20+	-		20+	-	
	48	9	++	.080	20+	++	.080	20+	++	.090	20+	++	.065	20++	++	.115	20+	++	.055	20++	++	.050	20++	+++	.090	20+	-	alk	20++	-	alk	20++	+++	.090	5	-	alk	20++	-	alk	20++	-	alk	20++	-	alk	20++	-	alk
		9	++	.080	20+	++	.080	20+	++	.080	20+	++	.070	20++	++	.115	20+	++	.060	20++	++	.045	20++	+++	.090	20+	-	alk	20++	-	alk	20++	+++	.100	5	-	alk	20++	-	alk	20++	-	alk	20++	-	alk	20++	-	alk
15°	24	0.5	-		0.5	-		0.2	-		0.2	-		5	-		0.2	-		9	-		9	-		5	-		0.5	-		5	-		5	-		0.5	-		0.5	-		5	-		0.5	-	
		0.5	-		0.5	-		0.2	-		0.2	-		5	-		0.2	-		9	-		9	-		5	-		0.5	-		5	-		5	-		0.5	-		0.5	-		5	-		0.5	-	
	48	20+	+		9	-		5	-		5	-		20++	+		5	-		20++	+		20++	+		9	-		9	-		20+	-		5	-		20++	-		20+	-		20+	-		20+	-	
		20+	+		9	±		5	-		5	-		20++	+		5	-		20++	+		20++	+		9	-		9	-		20+	-		5	-		20++	-		20+	-		20+	-		20+	-	
	72	20+	++	.060	20++	+	.040	20++	+	.070	20++	+	.060	20++	++	.075	20++	-	.035	20++	+	.020	20++	++	.080	9	-	alk	20++	-	alk	20++	++	.090	9	-	alk	20++	-	alk	20++	-	alk	20++	-	alk	20++	-	NA
		20+	++	.055	20++	+	.050	20++	+	.070	20++	+	.060	20++	++	.075	20++	-	.035	20++	+	.020	20++	++	.080	9	-	alk	20++	-	alk	20++	++	.090	9	-	alk	20++	-	alk	20++	-	alk	20++	-	alk	20++	-	NA
	144	0.2	-		0.5	-		5	-		5	-		20+	-		9	-		20++	-		20+	-		5	-		0.5	-		20+	-		0.5	-		5	-		5	-		20+	-		20+	-	
		0.2	-		0.5	-		5	-		5	-		20+	-		9	-		20++	-		20+	-		5	-		0.5	-		20+	-		0.5	-		5	-		5	-		20+	-		20+	-	
4°	192	5	-		5	-		20++	-		20+	-		20+	-		20++	-		20++	-		20+	-		9	-		20+	-		20++	-		0.5	-		9	-		9	-		20++	-		20++	-	
		5	-		5	-		20++	-		20+	-		20+	-		20++	-		20++	-		20+	-		9	-		20+	-		20++	-		0.5	-		9	-		9	-		20++	-		20++	-	
	240	20++	-	.040	20+	-	.040	20++	-	.020	20++	-	NA	20++	±	.040	20++	-	.010	20++	-	NA	20++	+	.020	20+	-	alk	20+	-	alk	20++	-	.030	20+	-	alk	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	alk
		20++	-	.040	20+	-	.035	20++	-	.020	20++	-	NA	20++	±	.030	20++	-	.010	20++	-	NA	20++	+	.020	20+	-	alk	20+	-	alk	20++	-	.030	20+	-	alk	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	alk

Abbreviation and symbols refer Table 1

Table 4 The fermentative ability of strain S12 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	-		0.1	-		-			-			0.5	+++		-			0.2	-		0.5	++	
		0.1	-		0.1	-		-			-			0.5	+++		-			0.2	-		0.5	++	
	48	0.5	+	.060	0.5	++	.090	-			-			20+	+++	.095	-			0.2	+	.045	0.5	+++	.045
		0.2	+	.055	0.5	++	.060	-			-			20+	+++	.085	-			0.2	+	.045	0.5	+++	.045
25°	24	0.5	+		0.5	++		5	+		5	+		20++	++		5	+		20++	++		20++	+++	
		0.5	+		0.5	++		5	+		5	+		20++	++		5	+		20++	++		20++	+++	
	48	20+	+++	.085	20+	+++	.070	20+	+++	.130	20+	+++	.085	20++	+++	.115	20+	+++	.075	20++	+++	.050	20++	+++	.090
		20+	+++	.110	20+	+++	.070	20+	+++	.130	20+	+++	.085	20++	+++	.115	20+	+++	.080	20++	+++	.060	20++	+++	.080
15°	24	0.5	+		0.5	+		0.2	-		0.2	-		9	+		0.2	-		9	+		9	+	
		0.5	+		0.5	+		0.2	-		0.2	-		9	+		0.2	-		9	+		9	+	
	48	20+	++		20+	++		9	+		9	++		20++	+		9	+		20++	+		20++	++	
		20+	++		20+	++		9	+		9	++		20++	+		9	+		20++	+		20++	++	
	72	20+	+++	.085	20++	+++	.130	20++	++	.120	20++	++	.080	20++	+++	.090	20++	+++	.065	20++	++	.030	20++	+++	.090
		20+	+++	.085	20++	+++	.100	20++	++	.120	20++	++	.070	20++	+++	.090	20++	+++	.055	20++	++	.030	20++	+++	.095
4°	144	0.5	+		0.5	+		9	-		5	-		20++	-		5	-		20+	-		9	-	
		0.5	+		0.5	+		9	-		5	-		20++	-		5	-		20+	-		9	-	
	192	9	++		20+	++		20+	++		20+	+		20+	+		20++	++		20+	+		20++	+	
		9	++		20+	++		20+	++		20+	+		20+	+		20++	++		20+	-		20++	+	
	240	20++	+++	.070	20++	++	.075	20++	+++	.100	20+	++	.065	20++	++	.060	20++	+++	.040	20++	+	.020	20++	+++	.065
		20++	+++	.070	20++	++	.070	20++	+++	.100	20+	++	.065	20++	++	.060	20++	+++	.050	20++	+	.020	20++	+++	.065

incubation temp.	incubation hours	lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.2	+		0.5	+		0.2	-		0.5	+		-			-			0.1	-		0.1	-	
		0.2	+		0.5	+		0.2	-		0.5	+		-			0.1	-		0.1	-		0.1	-	
	48	0.5	++	.070	0.5	++	.050	0.2	-	.050	0.5	++	.045	0.05	-	NA	0.05	-	.010	0.1	-	.010	0.1	-	.010
		0.5	++	.070	0.5	++	.050	0.2	-	.050	0.5	++	.045	0.05	-	NA	0.1	-	.020	0.1	-	.010	0.1	-	.010
25°	24	0.5	+		20+	++		20+	+		9	++		9	-		9	-		20+	-		20+	-	
		0.5	+		20+	++		20+	+		9	++		9	-		9	-		20+	±		20+	-	
	48	20++	++	.075	20++	+++	.125	20++	+++	.070	20++	+++	.080	20++	-	alk	20++	-	.025	20+	-	alk	20++	-	.010
		20++	++	.115	20++	+++	.110	20++	+++	.080	20++	+++	.100	20++	-	alk	20++	-	.025	20+	±	alk	20++	-	.010
15°	24	20+	+		9	+		5	-		20+	+		0.2	-		0.2	-		5	-		5	-	
		20+	+		9	+		5	-		20+	+		0.2	-		0.2	-		5	-		5	-	
	48	20++	+		20++	+		20+	++		20++	+		20++	-		20++	-		20+	-		20+	-	
		20++	+		20++	+		20+	++		20++	+		20++	-		20++	-		20+	±		20+	-	
	72	20++	++	.110	20++	++	.060	20++	+++	.130	20++	+++	.090	20++	-	NA	20++	-	.025	20++	-	alk	20++	-	NA
		20++	++	.090	20++	++	.075	20++	+++	.135	20++	+++	.080	20++	-	NA	20++	-	.035	20++	±	alk	20++	-	NA
4°	144	0.5	-		5	-		20++	-		5	-		5	-		0.5	-		20+	-		20+	-	
		0.5	+		5	-		20++	-		5	-		5	-		0.5	-		20+	-		20+	-	
	192	9	+		9	+		20++	+		9	+		9	-		9	-		20+	-		20+	-	
		9	+		9	+		20++	+		9	+		9	-		9	-		20+	-		20+	-	
	240	20++	+++	.080	20++	++	.035	20++	+++	.090	20++	++	.050	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA
		20++	+++	.070	20++	++	.035	20++	+++	.090	20++	++	.060	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 5 The fermentative ability of strain p5 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	+		0.2	+		-			-			0.5	-		-			0.2	++		20+	++	
		0.1	+		0.2	+		-			-			0.5	-		-			0.2	++		20+	++	
	48	0.5	+	.070	5	+++	.045	-			0.1	-	.020	0.5	+	.075	-			0.5	++	.080	20+	++	.055
		0.5	+	.070	5	+++	.045	-			0.1	-	.020	0.5	+	.085	-			0.5	++	.085	20+	++	.055
25°	24	0.5	++		0.5	++		5	+		0.5	+		20+	+		0.5	-		20++	+++		20+	++	
		0.5	++		0.5	++		5	+		0.5	+		20+	+		0.5	-		20++	+++		20+	++	
	48	20+	+++	.075	20+	+++	.080	20+	++	.100	20+	++	.075	20++	++	.105	20+	++	.055	20++	+++	.130	20++	+++	.075
		20+	+++	.075	20+	+++	.080	20+	++	.100	20+	++	.070	20++	++	.090	20+	++	.055	20++	+++	.125	20++	+++	.075
15°	24	0.5	+		0.5	±		0.1	-		0.1	-		5	-		0.1	-		5	-		0.5	±	
		0.5	+		0.5	±		0.1	-		0.1	-		5	-		0.1	-		5	-		0.5	±	
	48	20+	+		20+	++		9	+		5	±		20++	+		5	±		20++	++		20++	++	
		20+	+		20+	++		9	+		5	±		20+	+		5	±		20++	++		20++	++	
	72	20+	+++	.050	20++	++	.060	20++	++	.070	20++	+	.050	20++	+	.070	20++	+	.045	20++	++	.070	20++	++	.070
		20+	+++	.055	20++	++	.080	20++	++	.065	20++	+	.050	20++	+	.070	20++	+	.065	20++	++	.080	20++	++	.065
4°	144	0.5	+		0.5	+		9	-		9	-		20+	-		9	-		20+	-		9	-	
		0.5	+		0.5	+		9	-		9	-		20+	-		9	-		20+	-		9	-	
	192	9	+		20+	+		20++	-		20+	±		20+	-		20++	-		20+	-		20+	+	
		9	+		20+	+		20++	-		20+	±		20+	-		20++	-		20+	-		20+	±	
	240	20++	+	.070	20++	+	.050	20++	++	.075	20++	++	.045	20++	±	.050	20++	+	.030	20++	+	.050	20++		.050
		20++	+	.080	20++	+	.050	20++	++	.070	20++	++	.050	20++	±	.050	20++	+	.030	20++	+	.050	20++		.045

incubation temp.	incubation hours	lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.5	+		0.5	++		5	-		0.5	±		0.2	-		0.2	-		0.5	-		9	±	
		0.5	+		0.5	++		5	-		0.5	±		0.2	-		0.2	-		0.5	-		9	±	
	48	5	++	.095	9	++	.055	5	++	.060	9	+	.065	0.2	-	NA	0.5	-	.040	0.5	-	NA	9	+	.040
		5	++	.090	9	++	.055	5	++	.060	9	+	.065	0.2	-	NA	0.5	-	.035	0.5	-	NA	9	+	.035
25°	24	9	+		20+	++		20+	±		20+	±		9	±		9	+		20+	-		20+	±	
		9	+		20+	++		20+	±		20+	±		9	±		9	+		20+	-		20+	±	
	48	20++	+++	.110	20++	+++	.090	20++	+++	.090	20++	±	.040	20++	±	NA	20++	++	.015	20++	-	alk	20++	++	.030
		20++	+++	.120	20++	+++	.100	20++	+++	.090	20++	±	.050	20++	±	NA	20++	++	.015	20++	-	alk	20++	++	.030
15°	24	9	-		5	-		0.2	-		5	-		0.5	-		0.5	-		0.2	-		0.05	-	
		9	-		5	-		0.2	-		5	-		0.5	-		0.5	-		0.2	-		0.1	-	
	48	20+	+		20+	+		20+	-		20++	±		20+	-		20++	+		9	-		9	-	
		20+	+		20+	+		20+	-		20++	±		20+	±		20++	-		9	-		9	-	
	72	20++	+	.080	20+	++	.090	20++	+++	.070	20++	±	.060	20++	±	NA	20++	++	.015	20+	-	alk	20++	++	.040
		20++	+	.090	20+	++	.085	20++	+++	.070	20++	±	.060	20++	±	NA	20++	++	.025	20+	-	alk	20++	++	.045
4°	144	5	-		5	-		9	-		0.5	-		5	-		0.5	-		20+	-		20+	-	
		5	-		5	-		9	-		0.5	-		5	-		0.5	-		20+	-		20+	-	
	192	9	-		9	-		20++	-		20+	-		9	-		9	-		20+	-		20++	-	
		9	-		9	-		20++	-		20+	-		9	-		9	-		20+	-		20++	-	
	240	20++	-	.060	20++	+	.050	20++	-	.030	20++	-	.035	20++	-	alk	20++	-	NA	20++	-	alk	20++	-	.030
		20++	-	.060	20++	+	.050	20++	-	.030	20++	-	.040	20++	-	alk	20++	-	NA	20++	-	alk	20++	-	.020

Abbreviation and symbols refer Table 1

Table 6 The fermentative ability of strain p7 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	-		0.1	-		-			-			20+	-		-			9	+		20+	+		0.2	-		0.5	-		0.5	-		0.2	-		0.2	-		0.2	-		0.2	-		0.5	-	
		0.1	/		0.2	-		-			-			20+	-		-			9	+		20+	+		0/2	-		0.5	-		0.5	-		0.2	-		0.2	-		0.2	-		0.5	-	
	48	0.5	-	alk	5	-	NA	-			-			20+	-	.085	-			9	++	.035	20+	++	.045	0.5	-	alk	0.5	-	alk	0.5	-	alk	0.5	-	alk	0.2	-	alk	0.2	-	alk	0.2	-	NA	0.5	-	NA
		0.5	/	alk	5	-	NA	-			-			20+	-	.085	-			9	++	.035	20+	++	.050	0.5	-	alk	0.5	-	alk	0.5	-	alk	0.5	-	alk	0.2	-	alk	0.2	-	alk	0.2	-	NA	0.5	-	NA
25°	24	0.2	-		0.2	-		0.1	-		0.1	-		20++	+		0.1	-		20+	++		20+	+		0.2	-		0.5	-		9	±		0.2	-		9	-		9	-		0.2	-		0.2	-	
		0.2	-		0.2	-		0.1	-		0.1	-		20++	+		0.1	-		20+	++		20+	+		0.2	-		0.5	-		9	±		0.2	-		9	-		9	-		0.2	-		0.2	-	
	48	0.5	-	alk	5	-	NA	5	-	alk	5	-	alk	20++	++	.125	5	-	NA	20++	++	.075	20++	++	.070	5	-	alk	5	-	alk	20+	±	.065	5	-	alk	20++	-	alk	20++	-	alk	0.2	-	alk	0.5	-	NA
		0.5	-	alk	5	-	NA	5	-	alk	5	-	alk	20++	++	.115	5	-	NA	20++	++	.080	20++	++	.070	5	-	alk	5	-	alk	20+	±	.045	5	-	alk	20++	-	alk	20++	-	alk	0.2	-	alk	0.5	-	NA
15°	24	0.1	-		0.1	-		0.1	-		0.1	-		0.2	-		0.1	-		0.1	-		0.2	-		0.5	-		5	-		0.1	-		0.5	-		0.5	-		0.2	-		0.05	-		0.05	-	
		0.1	-		0.1	-		0.1	-		0.1	-		0.2	-		0.1	-		0.1	-		0.2	-		0.5	-		5	-		0.1	-		0.5	-		0.2	-		0.05	-		0.05	-	
	48	0.2	-		0.5	-		0.1	-		0.2	-		20+	+		0.1	-		20+	+		20+	±		5	-		5	-		0.5	-		0.5	-		20++	-		20+	-		0.5	-		0.2	-	
		0.2	-		0.5	-		0.1	-		0.2	-		20+	+		0.1	-		20+	+		20+	-		5	-		5	-		0.5	-		0.5	-		20++	-		20+	-		0.5	-		0.2	-	
	72	5	-	NA	0.5	-	.020	0.5	-	alk	0.5	-	NA	20++	+	.070	0.2	-	alk	20++	++	.020	20++	++	.065	9	-	alk	5	-	alk	20++	++	.100	0.5	-	alk	20++	-	alk	20++	-	alk	20+	-	alk	20+	-	NA
		5	-	NA	0.5	-	.020	0.5	-	alk	0.5	-	NA	20++	+	.070	0.2	-	alk	20++	++	.020	20++	++	.065	9	-	alk	5	-	alk	20++	++	.100	0.5	-	alk	20++	-	alk	20++	-	alk	20+	-	alk	20+	-	NA
4°	144	-			-			0.1	-		0.1	-		0.2	-		0.1	-		0.2	-		0.2	-		0.2	-		0.1	-		0.2	-		0.1	-		0.5	-		5	-		0.2	-		0.5	-	
		-			-			0.1	-		0.1	-		0.2	-		0.1	-		0.2	-		0.2	-		0.2	-		0.1	-		0.2	-		0.1	-		0.5	-		5	-		0.2	-		0.5	-	
	192	0.1	-		0.5	-		0.1	-		0.2	-		5	-		0.2	-		5	-		0.5	-		0.2	-		0.2	-		5	-		0.1	-		5	-		9	-		5	-		5	-	
		0.1	-		0.5	-		0.1	-		0.2	-		5	-		0.2	-		5	-		0.5	-		0.2	-		0.2	-		5	-		0.1	-		5	-		9	-		5	-		5	-	
	240	0.5	-	NA	20++	-	.020	0.2	-	NA	0.5	-	NA	20++	-	.035	5	-	NA	20++	-	NA	20++	-	.020	0.2	-	alk	0.2	-	NA	20+	-	.040	0.5	-	alk	9	-	alk	20++	-	alk	20+	-	alk	9	-	alk
		0.5	-	NA	20++	-	.015	0.2	-	NA	0.5	-	NA	20++	-	.035	5	-	NA	20++	-	NA	20++	-	.025	0.2	-	alk	0.2	-	NA	20+	-	.040	0.5	-	alk	9	-	alk	20++	-	alk	20+	-	alk	9	-	alk

Abbreviation and symbols refer Table 1

Table 7 The fermentative ability of strain p8 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	±		0.2	-		-			-			0.1	-		-			0.1	-		0.1	-	
		0.2	±		0.2	±		-			-			0.1	-		-			0.1	-		0.1	-	
	48	0.5	++	.070	0.5	++	.060	-			-			0.2	-	NA	-			0.5	-	NA	0.2	-	NA
		0.5	++	.070	0.5	++	.060	-			-			0.2	-	NA	-			0.5	-	NA	0.2	-	NA
25°	24	0.5	+		0.5	+		0.2	-		0.2	-		9	-		0.2	-		9	-		9	-	
		0.5	+		0.5	+		0.2	-		0.2	-		9	-		0.2	-		9	-		9	-	
	48	20+	+++	.100	20+	++	.085	20+	+	.090	9	+	.065	20++	-	alk	20+	-	.060	20++	-	NA	20++	-	alk
		20+	+++	.100	20+	++	.100	20+	+	.080	9	+	.060	20++	-	alk	20+	-	.065	20++	-	NA	20++	-	alk
15°	24	0.05	-		0.1	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-	
		0.1	-		0.2	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-	
	48	5	+		5	+		0.2	-		0.2	-		20++	-		0.2	-		20++	-		20+	-	
		5	+		5	+		0.2	-		0.2	-		20++	-		0.2	-		20++	-		20+	-	
	72	20+	++	.105	20++	++	.070	9	-	.060	9	+	.050	20++	-	alk	5	-	.030	20++	-	alk	20++	-	alk
		20+	++	.095	20++	++	.060	9	-	.055	5	-	.040	20++	-	alk	5	-	.030	20++	-	alk	20++	-	alk
4°	144	0.2	-		0.2	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-	
		0.2	-		0.2	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-	
	192	5	-		5	-		0.5	-		0.5	-		9	-		5	-		20+	-		9	-	
		5	-		5	-		0.5	-		0.5	-		9	-		5	-		20+	-		9	-	
	240	20++	-	.050	20+	±	.040	9	-	.025	5	-	.030	20+	-	alk	5	-	.010	20++	-	NA	20++	-	alk
		20++	-	.050	20+	±	.030	9	-	.020	5	-	.030	20+	-	alk	5	-	.010	20++	-	NA	20++	-	alk

incubation temp.	incubation hours	lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.2	+		0.2	+		0.1	-		0.2	+		-			-			0.2	-		0.1	-	
		0.2	+		0.2	+		0.1	-		0.2	+		-			-			0.2	-		0.1	-	
	48	9	+	.055	5	++	.070	0.5	-	.030	5	+	.025	0.1	-	NA	0.1	-	NA	0.2	-	NA	0.2	-	NA
		9	+	.055	5	++	.060	0.5	-	.020	5	+	.035	0.1	-	NA	0.1	-	NA	0.2	-	NA	0.2	-	NA
25°	24	5	++		5	++		20+	+		5	+		9	-		5	-		5	-		0.1	-	
		5	++		5	++		20+	+		5	+		9	-		5	-		5	-		0.1	-	
	48	20++	+++	.110	20++	+++	.095	20++	+++	.110	20++	++	.070	20++	-	alk	20+	-	alk	9	-	alk	0.2	-	NA
		20++	+++	.130	20++	+++	.090	20++	+++	.100	20++	++	.065	20++	-	alk	20+	-	alk	9	-	alk	0.2	-	NA
15°	24	0.5	-		0.5	-		0.05	-		0.5	-		0.1	-		0.2	-		0.1	-		0.05	-	
		0.5	-		0.5	-		0.05	-		0.5	-		0.1	-		0.2	-		0.1	-		0.05	-	
	48	20+	+		20+	+		9	-		9	+		0.5	-		5	-		5	-		0.5	-	
		20+	+		20+	+		9	-		9	+		0.5	-		5	-		5	-		0.5	-	
	72	20++	++	.150	20++	++	.070	20++	+++	.190	20++	+	.070	9	-	alk	9	-	alk	20++	-	alk	20++	-	NA
		20++	++	.140	20++	++	.090	20++	+++	.150	20++	+	.080	9	-	alk	9	-	alk	20++	-	alk	20++	-	NA
4°	144	0.2	-		0.1	-		0.5	-		0.1	-		0.05	-		0.1	-		0.5	-		0.5	-	
		0.5	-		0.1	-		0.5	-		0.1	-		0.05	-		0.1	-		0.5	-		0.5	-	
	192	5	-		5	-		20++	±		0.5	-		0.1	-		0.1	-		20+	-		20+	-	
		5	-		5	-		20++	±		0.5	-		0.1	-		0.1	-		20+	-		20+	-	
	240	20++	-	.040	20+	±	.020	20++	±	.050	9	-	.010	0.1	-	NA	0.1	-	NA	20++	-	alk	20++	-	NA
		20++	-	.030	20+	±	.015	20++	±	.050	20+	-	.010	0.1	-	NA	0.1	-	NA	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 8 The fermentative ability of strain p9 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			arabinose			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.05	-		-			-			-			0.5	-		-			0.5	+		9	+		0.2	-		0.1	-		0.2	-		0.1	-		?			0.1	-		0.05	-		-		
		0.05	-		-			-			-			0.5	-		-			0.5	+		9	+		0.2	-		0.1	-		0.2	-		0.1	-		0.05	-		0.1	-		0.05	-		-		
	48	0.2	-	alk	0.1	-	alk	-			-			5	+	.070	-			0.5	+++	.085	20+	++	.035	0.2	-	NA	0.2	-	alk	0.2	-	.060	0.1	-	alk	0.1	-	.010	0.2	-	.050	0.2	-	NA	0.5	-	NA
		0.2	-	alk	0.1	-	alk	-			-			5	+	.080	-			0.5	+++	.085	20+	++	.040	0.2	-	NA	0.2	-	alk	0.2	-	.060	0.1	-	alk	0.2	-	.020	0.2	-	.050	0.2	-	NA	0.5	-	NA
25°	24	0.5	-		0.5	-		0.2	-		0.2	-		20+	+		0.2	-		20+	++		20+	+		0.5	-		0.5	-		0.5	-		0.2	-		9	-		9	-		0.2	-		0.05	-	
		0.5	-		0.5	-		0.2	-		0.2	-		20+	+		0.2	-		20+	++		20+	+		0.5	-		0.5	-		0.5	-		0.2	-		9	-		9	-		0.2	-		0.05	-	
	48	9	-	.015	20+	-	.015	20+	++	.080	20+	+	.055	20++	++	.125	9	+	.065	20++	++	.125	20++	+++	.085	5	-	alk	5	-	alk	9	-	.055	0.5	-	alk	20++	-	NA	20++	-	.025	0.5	-	alk	0.5	-	.035
		9	-	.015	20+	-	.020	20+	++	.090	20+	+	.055	20++	++	.125	9	±	.060	20++	++	.125	20++	+++	.070	5	-	alk	9	-	alk	9	-	.060	0.5	-	alk	20++	-	NA	20++	-	.025	0.5	-	alk	0.5	-	.040
15°	24	-			-			0.05	-		0.05	-		0.2	-		0.05	-		0.2	-		0.2	-		0.5	-		0.5	-		-			0.2	-		0.5	-		0.5	-		-			-		
		-			-			0.05	-		0.05	-		0.2	-		0.05	-		0.2	-		0.2	-		0.5	-		0.5	-		-			0.2	-		0.5	-		0.5	-		-			-		
	48	0.5	-		5	-		0.5	-		0.5	-		20+	+		0.5	-		9	+		20+	+		0.5	-		0.5	-		0.5	-		0.2	-		20++	-		20++	-		0.5	-		5	-	
		0.5	-		5	-		0.5	-		0.5	-		20+	+		0.5	-		9	+		20+	+		0.5	-		0.5	-		0.5	-		0.2	-		20++	-		20++	-		0.5	-		5	-	
	72	20+	-	.010	20+	-	.030	20+	+	.070	20+	+	.060	20++	+	.070	20+	-	.030	20++	+	.060	20++	+	.060	5	-	alk	5	-	alk	20+	-	.035	0.5	-	alk	20++	-	NA	20++	-	NA	20+	-	alk	20+	-	.030
		20+	-	.010	20+	-	.035	20+	+	.060	20+	+	.060	20++	+	.070	20+	-	.030	20++	+	.065	20++	+	.060	5	-	alk	5	-	alk	20+	-	.035	0.5	-	alk	20++	-	NA	20++	-	NA	20+	-	alk	20+	-	.030
4°	144	0.1	-		0.1	-		0.1	-		0.1	-		0.5	-		0.1	-		0.2	-		0.5	-		0.1	-		0.1	-		5	-		-			0.5	-		5	-		9	-		0.5	-	
		0.1	-		0.1	-		0.1	-		0.1	-		0.5	-		0.1	-		0.2	-		0.5	-		0.1	-		0.1	-		5	-		-			0.5	-		5	-		9	-		0.5	-	
	192	0.1	-		5	-		20+	-		5	-		9	-		0.5	-		5	-		5	-		0.1	-		0.1	-		5	-		?			5	-		5	-		9	-		20++	-	
		0.1	-		5	-		20+	-		5	-		9	-		0.5	-		5	-		5	-		0.1	-		0.1	-		5	-		?			5	-		5	-		9	-		20++	-	
	240	5	-	.010	9	-	.020	20+	-	.020	5	-	.020	20++	-	.020	9	-	alk	20++	-	.015	20++	-	.030	0.2	-	alk	0.1	-	alk	20+	-	.020	?			20+	-	alk	20+	-	alk	20+	-	alk	20+	-	alk	20++	-	NA
		5	-	.010	9	-	.030	20+	-	.020	5	-	.020	20++	-	.020	9	-	alk	20++	-	.020	20++	-	.030	0.2	-	alk	0.1	-	alk	20+	-	.020	?			20+	-	alk	20+	-	alk	20+	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 9 The fermentative ability of strain p13 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C		0.05	-		0.1	-		-			-			0.5	+		-			0.2	+		0.5	+		0.1	-		0.2	-		0.1	-		0.1	-		?			0.1	-		0.1	-	
	24	0.05	-		0.1	-		-			-			0.5	+		-			0.2	+		0.5	+		0.1	-		0.2	-		0.1	-		0.1	-		?			0.1	-		0.1	-	
		0.5	-	.040	0.5	-	.030	-			-			0.5	+	.075	-			0.2	++	.085	20+	++	.045	0.5	-	alk	0.5	-	alk	0.5	-	.040	0.2	-	alk	0.2	-	alk	0.2	-	alk	0.2	-	NA
	48	0.5	-	.040	0.5	-	.030	-			-			0.5	+	.080	-			0.2	++	.085	20+	++	.045	0.5	-	alk	0.5	-	alk	0.5	-	.045	0.2	-	alk	0.2	-	alk	0.2	-	alk	0.2	-	NA
		0.5	-		5	-		0.2	-		0.5	-		20+	+		0.2	-		20+	+		20+	++		0.5	-		5	-		20+	+		0.5	-		9	-		9	-		9	-	
	24	0.5	-		5	-		0.2	-		0.5	-		20+	+		0.2	-		20+	+		20+	++		0.5	-		5	-		20+	++		0.5	-		9	-		9	-		9	-	
25°																																														
		20+	-	.060	9	-	.040	20+	++	.090	20+	++	.065	20++	++	.120	20++	+	.045	20++	+++	.095	20++	+++	.080	9	-	alk	9	-	alk	20++	+++	.100	9	-	alk	20+	-	alk	20+	-	alk	20+	-	alk
	48	20+	-	.065	9	-	.045	20+	++	.080	20+	++	.070	20++	++	.115	20++	+	.050	20++	+++	.105	20++	+++	.080	9	-	alk	9	-	alk	20++	+++	.100	9	-	alk	20+	-	alk	20+	-	alk	20+	-	alk
		0.5	-		5	-		0.1	-		0.1	-		9	-		0.1	-		5	-		5	-		9	-		5	-		5	-		5	-		0.5	-		0.5	-		5	-	
	24	0.5	-		5	-		0.1	-		0.1	-		9	-		0.1	-		5	-		5	-		9	-		5	-		5	-		5	-		0.5	-		0.5	-		5	-	
15°		5	-		9	-		5	-		5	-		20++	+		5	-		20++	+		20++	-		9	-		5	-		20+	-		5	-		20+	-		20++	-		9	-		20+	-	
	48	5	-		9	-		5	-		5	-		20++	+		5	-		20++	+		20++	-		9	-		5	-		20+	-		5	-		20+	-		20++	-		9	-		20+	-	
		20+	-	.050	20+	-	.040	20++	-	.060	20+	-	.050	20++	++	.065	20+	-	.030	20++	++	.010	20++	+	.070	20+	-	alk	20+	-	alk	20++	++	.100	5	-	alk	20++	-	alk	20++	-	NA	20+	-	alk	20++	-	alk
	72	20+	-	.050	20+	-	.050	20++	-	.055	20+	-	.045	20++	++	.070	20+	-	.030	20++	++	.015	20++	+	.065	20+	-	alk	20+	-	alk	20++	++	.100	5	-	alk	20++	-	alk	20++	-	NA	20+	-	alk	20++	-	alk
		0.5	-		0.5	-		5	-		0.5	-		0.5	-		0.5	-		0.5	-		0.5	-		5	-		5	-		5	-		0.5	-		5	-		5	-		5	-		9	-	
	144	0.5	-		0.5	-		5	-		0.5	-		0.5	-		0.5	-		0.5	-		0.5	-		5	-		5	-		5	-		0.5	-		5	-		5	-		5	-		9	-	
		9	-		5	-		20+	-		20+	-		20+	-		20++	-		20+	-		20+	-		9	-		5	-		20+	-		5	-		9	-		9	-		20+	-		20++	-	
4°	192	9	-		5	-		20+	-		20+	-		20+	-		20++	-		20+	-		20+	-		9	-		5	-		20+	-		5	-		9	-		9	-		20+	-		20++	-	
		20++	-	.030	20+	-	.035	20++	-	alk	20+	-	alk	20++	-	.015	20++	-	.020	20++	-	NA	20++	-	NA	20++	-	alk	20++	-	alk	20++	-	NA	20+	-	alk	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA
	240	20++	-	.030	20+	-	.040	20++	-	alk	20+	-	alk	20++	-	.020	20++	-	.020	20++	-	NA	20++	-	NA	20++	-	alk	20++	-	alk	20++	-	NA	20+	-	alk	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 10 The fermentative ability of strain p15[I] on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	-		0.1	-		-			-			0.5	+		-			0.2	-		0.5	+		0.2	-		0.2	+		0.5	-		0.1	+		0.1	-		0.1	-		0.2	-		0.2	-	
		0.1	-		0.1	-		-			-			0.5	+		-			0.2	-		0.5	±		0.5	+		0.2	+		0.5	-		0.2	+		0.1	-		0.1	-		0.2	-		0.2	-	
	48	0.5	-	.040	0.5	-	.040	-			-			0.5	+	.065	-			0.5	+	.035	5	+	.055	5	+	.050	0.5	+	.040	0.5	-	.060	5	+	.030	0.1	-	NA	0.1	-	.030	0.2	-	NA	0.2	-	.030
		0.5	-	.040	0.5	-	.040	-			-			0.5	+	.060	-			0.5	±	.035	5	+	.055	5	+	.050	0.5	+	.040	0.5	-	.060	5	+	.020	0.1	-	NA								
25°	24	0.5	-		0.5	-		0.5	-		0.2	-		20+	+		0.2	-		20+	+		20+	+		9	+		5	+		5	-		5	+		0.5	-		0.5	-		0.5	-		0.5	-	
		0.5	-		0.5	-		0.5	-		0.2	-		20+	+		0.2	-		20+	+		20+	+		9	+		5	+		5	-		5	+		0.5	-		0.5	-		9	-	.020	20+	-	alk	0.5	-	.050
		9	-	.050	5	-	.045	20+	-	.100	9	-	.055	20++	++	.095	20+	-	.075	20++	+	.045	20++	++	.080	20++	+	.070	20++	-	.100	20++	+	.040	5	-	alk	9	-	.015	20+	-	alk	0.5	-	.050
	48	9	-	.055	5	-	.055	20+	-	.100	9	-	.055	20++	++	.095	20+	-	.075	20++	+	.050	20++	++	.080	20++	+	.065	20++	-	.100	20++	+	.045	5	-	alk												
15°	24	0.2	-		0.5	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-		0.5	-		0.5	-		0.2	-		0.2	-		0.1	-		0.1	-		0.2	-		0.5	-	
		0.2	-		0.2	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-		0.5	-		0.5	-		0.2	-		0.2	-		0.1	-		0.1	-		0.2	-		0.5	-	
		0.2	-		0.5	-		0.5	-		0.5	-		9	-		0.2	-		20++	-		5	-		9	±		20++	-		9	-		0.2	-		0.2	-		0.5	-	.025	20++	-	NA	20++	-	.020
	48	0.2	-		0.5	-		0.5	-		0.5	-		9	-		0.2	-		20++	-		5	-		9	±		20++	-		9	-		0.2	-		0.2	-		0.5	-	.020	20++	-	NA	20++	-	.020
		9	+	.070	9	+	.050	5	-	.070	9	-	.050	20++	+	.115	5	-	.035	20++	+	.060	9	+	.050	20++	+	.115	20++	+	.060	20++	-	.085	9	+	.070	0.2	-	NA								
	72	9	+	.080	5	±	.050	5	-	.080	9	-	.050	20++	+	.120	5	-	.035	20++	+	.060	9	+	.050	20++	+	.140	20++	+	.070	20++	-	.090	9	+	.080	0.2	-	NA								
		0.2	-		0.2	-		0.2	-		0.1	-		0.2	-		0.1	-		0.2	-		0.2	-		0.2	-		0.2	-		20+	-		0.2	-		0.1	-		0.1	-		20+	-		0.5	-	
		0.2	-		0.2	-		0.2	-		0.1	-		0.2	-		0.1	-		0.2	-		0.2	-		0.2	-		0.2	-		20+	-		0.2	-		0.1	-		0.1	-		20+	-		0.5	-	
	144	0.2	-		0.2	-		0.2	-		0.1	-		0.2	-		0.1	-		0.2	-		0.5	-		0.5	-		5	-		20++	-		5	-		0.2	-		0.2	-		20+	-		20++	-	
		5	-		0.5	-		0.5	-		0.2	-		20+	-		0.2	-		9	-		5	-		0.5	-		5	-		20++	-		5	-		0.2	-		0.2	-		20+	-		20++	-	
4°	192	5	-		0.5	-		0.5	-		0.2	-		20+	-		0.2	-		9	-		5	-		0.5	-		5	-		20++	-		5	-		0.2	-		0.2	-		20++	-		20++	-	
		9	-	.060	0.5	-	.035	20++	-	.080	9	-	.020	20++	-	.020	5	-	.020	20++	-	NA	20++	-	NA	20++	-	.035	20++	-	.020	20++	-	.060	9	-	NA	0.2	-	NA	0.2	-	.020	20++	-	alk	20++	-	NA
	240	9	-	.060	0.5	-	.035	20++	-	.070	9	-	.020	20++	-	.020	5	-	.020	20++	-	NA	20++	-	NA	20++	-	.030	20++	-	.025	20++	-	.060	9	-	NA	0.2	-	NA	0.2	-	.020	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 11 The fermentative ability of strain p19 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	-			-			-			-			0.1	-		-			0.1	-		-			0.1	-	
		-			-			-			-			0.1	-		-			0.1	-		-			0.1	-	
	48	-			-			-			-			0.2	++	.055	-			0.2	+	.080	0.2	++	.045	0.5	-	NA
		-			-			-			-			0.2	++	.055	-			0.1	+	.075	0.2	++	.045	0.5	-	NA
25°	24	0.5	-		0.5	-		0.05	-		0.1	-		5	-		0.05	-		9	+		9	-		0.2	-	
		0.5	-		0.5	-		0.05	-		0.1	-		5	-		0.05	-		9	+		9	-		0.2	-	
	48	20+	-	.015	20+	-	.020	9	+	.090	9	+	.050	20+	-	alk	20+	+	.050	20++	+++	.050	20+	-	NA	9	-	alk
		20+	-	.015	20+	-	.020	9	+	.090	9	+	.050	20+	-	alk	20+	+	.050	20++	+++	.055	20+	-	NA	9	-	alk
15°	24	-			-			0.05	-		0.05	-		0.2	-		0.05	-		0.2	-		-			0.2	-	
		-			-			0.05	-		0.05	-		0.2	-		0.05	-		0.2	-		-			0.2	-	
	48	9	-		5	-		0.2	-		0.5	-		0.5	-		0.5	-		0.5	-		5	-		0.5	-	
		9	-		5	-		0.2	-		0.5	-		0.5	-		0.5	-		0.5	-		5	-		0.5	-	
	72	20+	-	.020	20+	-	.030	20+	+	.080	20+	+	.055	5	-	alk	9	-	.025	5	-	alk	20+	-	.030	5	-	alk
		20+	-	.020	20+	-	.020	20+	+	.075	20+	+	.055	5	-	alk	9	-	.025	5	-	alk	20+	-	.030	5	-	alk
4°	144	0.1	-		0.2	-		0.5	-		5	-		0.1	-		5	-		0.1	-		0.2	-		0.1	-	
		0.1	-		0.2	-		0.5	-		5	-		0.1	-		5	-		0.1	-		0.2	-		0.1	-	
	192	0.5	-		0.5	-		20+	-		20+	-		9	-		20+	-		5	-		5	-		0.1	-	
		0.5	-		0.5	-		20+	-		20+	-		9	-		20+	-		5	-		5	-		0.1	-	
	240	20+	-	.020	20++	-	.020	20++	-	.010	20++	-	NA	20++	-	alk	20++	-	.020	20++	-	NA	20++	-	alk	0.2	-	NA
		20+	-	.020	20++	-	.020	20++	-	.010	20++	-	NA	20++	-	alk	20++	-	.020	20++	-	NA	20++	-	alk	0.2	-	NA

incubation temp.	incubation hours	maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.2	-		0.1	-		0.1	-		0.1	-		0.1	-		0.1	-		0.2	-	
		0.2	-		0.1	-		0.1	-		0.1	-		0.1	-		0.1	-		0.2	-	
	48	0.5	-	alk	0.2	-	.040	0.2	-	NA	0.1	±	NA	0.1	-	.020	0.1	-	alk	0.2	-	NA
		0.5	-	alk	0.2	-	.035	0.2	-	NA	0.1	-	NA	0.1	±	.020	0.1	-	alk	0.2	-	NA
25°	24	0.5	-		0.5	±		0.2	-		20+	-		9	+		5	-		0.1	-	
		0.5	-		0.5	±		0.2	-		20+	-		9	+		5	-		0.1	-	
	48	9	-	alk	20++	+++	.080	5	-	alk	20++	+	NA	20++	++	.015	20+	-	alk	0.2	-	alk
		9	-	alk	20++	+++	.080	5	-	alk	20++	+	NA	20++	++	.020	20+	-	alk	0.2	-	alk
15°	24	0.2	-		-			-			-			-			-			-		
		0.2	-		-			-			-			-			-			-		
	48	0.5	-		5	-		9	-		9	-		9	-		5	-		0.5	-	
		0.5	-		5	-		9	-		9	-		9	-		5	-		0.5	-	
	72	5	-	alk	20++	++	.120	20+	-	.020	20++	±	NA	20++	+	.025	20++	-	alk	20++	-	NA
		5	-	alk	20++	++	.140	20+	-	.020	20++	±	NA	20++	+	.020	20++	-	alk	20++	-	NA
4°	144	0.1	-		0.5	-		0.1	-		0.2	-		0.1	-		0.5	-		0.5	-	
		0.1	-		0.5	-		0.1	-		0.2	-		0.1	-		0.5	-		0.5	-	
	192	0.1	-		20+	-		0.1	-		0.5	-		0.5	-		20+	-		20++	-	
		0.1	-		20+	-		0.1	-		0.5	-		0.5	-		20+	-		20++	-	
	240	0.2	-	alk	20++	±	.045	0.2	-	alk	20+	-	NA	9	-	.020	20+	-	alk	20++	-	NA
		0.2	-	alk	20++	±	.045	0.2	-	alk	20+	-	NA	9	-	.020	20+	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 12 The fermentative ability of strain p152 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	-		0.1	-		-			-			0.2	-		-			0.2	-		0.1	-	
		0.1	-		0.1	-		-			-			0.2	-		-			0.2	-		0.1	-	
	48	0.5	-	.070	0.5	-	.030	-			-			0.5	-	alk	-			0.5	-	alk	0.2	-	alk
		0.5	-	.060	0.5	-	.030	-			-			0.5	-	alk	-			0.5	-	alk	0.2	-	alk
25°	24	0.5	-		0.5	-		0.2	-		0.2	-		0.5	-		0.2	-		0.5	-		0.5	-	
		0.5	-		0.2	-		0.2	-		0.2	-		0.5	-		0.2	-		0.5	-		0.5	-	
	48	9	-	.060	20+	-	.030	20+	+	.080	20+	++	.065	9	-	alk	20+	+	.055	9	-	NA	20+	-	alk
		9	-	.060	20+	-	.050	20+	+	.070	20+	++	.065	9	-	alk	20+	+	.055	9	-	NA	20+	-	alk
15°	24	0.05	-		0.2	-		0.1	-		0.05	-		5	-		0.05	-		0.5	-		0.2	-	
		0.05	-		0.2	-		0.1	-		0.05	-		5	-		0.05	-		0.5	-		0.2	-	
	48	0.5	-		9	-		0.2	-		0.2	-		5	-		0.2	-		5	-		5	-	
		0.5	-		9	-		0.2	-		0.2	-		5	-		0.2	-		5	-		5	-	
	72	9	-	.085	9	-	.040	9	+	.060	9	+	.060	9	-	alk	5	-	.040	9	-	alk	9	-	.040
		9	-	.070	9	-	.040	9	+	.060	9	+	.060	9	-	alk	5	-	.040	9	-	alk	9	-	.040
4°	144	0.1	-		-			0.1	-		0.1	-		0.5	-		0.1	-		0.1	-		0.5	-	
		0.1	-		-			0.1	-		0.1	-		0.5	-		0.1	-		0.1	-		0.5	-	
	192	0.2	-		0.2	-		5	-		5	-		9	-		0.5	-		0.5	-		9	-	
		0.2	-		0.2	-		5	-		5	-		9	-		0.5	-		0.5	-		9	-	
	240	0.5	-	NA	0.5	-	NA	20++	-	.030	20+	-	NA	20++	-	alk	9	-	.020	20+	-	alk	20+	-	alk
		0.5	-	NA	0.5	-	NA	20++	-	.035	20+	-	NA	20++	-	alk	9	-	.020	20+	-	alk	20+	-	alk

incubation temp.	incubation hours	lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	-		0.1	-		0.2	-		0.1	-		0.2	-		0.1	-		0.2	-		0.2	-	
		0.1	-		0.1	-		0.2	-		0.1	-		0.2	-		0.1	-		0.2	-		0.2	-	
	48	0.2	-	alk	0.2	-	alk	0.2	+	.080	0.1	-	NA	0.5	+	NA	0.2	-	.030	0.5	-	NA	5	±	.025
		0.2	-	alk	0.2	-	alk	0.2	±	.075	0.1	-	NA	0.5	±	NA	0.2	-	.030	0.5	-	NA	5	±	.025
25°	24	0.5	-		0.5	-		0.5	-		0.2	-		9	+		20+	+		5	-		0.5	-	
		0.5	-		0.5	-		0.5	-		0.2	-		9	-		20+	+		5	-		0.5	-	
	48	9	-	alk	9	-	alk	20++	++	.090	5	-	alk	20++	+	NA	20++	++	.025	20++	-	alk	20++	++	.030
		9	-	alk	9	-	alk	20++	++	.090	5	-	alk	20++	+	NA	20++	++	.025	20++	-	alk	20++	++	.025
15°	24	0.5	-		0.5	-		-			0.05	-		0.1	-		0.1	-		-			-		
		0.5	-		0.5	-		-			0.05	-		0.1	-		0.1	-		-			-		
	48	0.5	-		0.5	-		5	-		0.5	-		20+	-		20+	+		5	-		0.2	-	
		0.5	-		0.5	-		5	-		0.5	-		20+	-		20+	+		5	-		0.2	-	
	72	9	-	alk	9	-	alk	20++	±	.050	9	-	.085	20++	+	NA	20++	++	.025	20+	-	alk	20++	±	.030
		9	-	alk	9	-	alk	20++	±	.050	9	-	.070	20++	+	NA	20++	++	.030	20+	-	alk	20++	±	.035
4°	144	0.1	-		0.1	-		5	-		0.1	-		0.2	-		0.2	-		5	-		0.5	-	
		0.1	-		0.1	-		5	-		0.1	-		0.2	-		0.2	-		5	-		0.5	-	
	192	0.2	-		0.2	-		20+	-		0.1	-		5	-		0.5	-		20+	-		20++	-	
		0.2	-		0.2	-		20+	-		0.1	-		5	-		0.5	-		20+	-		20++	-	
	240	0.2	-	alk	5	-	alk	20++	-	.030	0.5	-	alk	20+	-	NA	20+	-	.030	20++	-	alk	20++	-	NA
		0.2	-	alk	5	-	alk	20++	-	.030	0.5	-	alk	20+	-	NA	20+	-	.020	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 13 The fermentative ability of strain p181 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C		0.05	-		0.05	-		-			-			0.5	+		-			0.5	+		0.5	++		0.5	++		0.5	+		0.1	-		0.5	+		-			0.1	-		0.2	-		?		
	24	0.05	-		0.05	-		-			-			0.5	+		-			0.5	±		0.5	++		0.5	++		0.5	+		0.1	-		0.5	+		-			0.1	-		0.2	-		?		
		0.1	-	.010	0.1	-	.010	-			-			0.5	++	.065	-			5	++	.095	9	+++	.040	9	+++	.085	9	++	.050	0.5	-	.040	5	++	.055	0.05	-	NA	0.1	-	.020	0.5	-	NA	0.2	-	alk
	48	0.1	-	.010	0.1	-	.010	-			-			0.5	++	.065	-			5	++	.095	9	+++	.040	9	+++	.085	9	++	.060	0.5	-	.030	5	++	.050	0.1	-	NA	0.1	-	.020	0.5	-	NA	0.2	-	alk
		5	-		0.5	-		0.2	-		0.2	-		20+	+		0.2	-		20+	+		20+	++		9	+		5	+		20+	+		9	+		9	-		9	-		20+	-		20+	-	
25°	24	5	-		0.5	-		0.2	-		0.2	-		20+	+		0.2	-		20+	+		20+	++		9	+		5	+		20+	+		9	+		9	-		9	-		20+	-		20+	-	
		20+	-	.010	20+	-	.015	9	-	NA	9	-	alk	20++	++	.130	9	-	NA	20++	+++	.105	20++	+++	.080	20+	+++	.080	20++	++	.100	20++	++	.110	20++	++	.135	20++	-	alk	20++	-	.025	20++	-	alk	20++	-	alk
	48	20+	-	.015	20+	-	.015	9	-	NA	9	-	alk	20++	++	.125	9	-	NA	20++	+++	.105	20++	+++	.080	20+	+++	.085	20++	++	.095	20++	++	.110	20++	++	.090	20++	-	alk	20++	-	.025	20++	-	alk	20++	-	alk
		0.2	-		0.5	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-		0.5	-		0.5	-		0.5	-		0.2	-		0.2	-		0.2	-		0.5	-		0.2	-	
	24	0.2	-		0.5	-		0.1	-		0.1	-		0.5	-		0.1	-		0.5	-		0.5	-		0.5	-		0.5	-		0.5	-		0.2	-		0.2	-		0.2	-		0.5	-		0.2	-	
		5	-		9	-		0.2	-		0.2	-		9	-		0.5	-		9	±		9	-		9	+		9	±		20+	+		5	-		20++	-		20+	-		20+	-		20+	-	
15°	48	5	-		9	-		0.2	-		0.2	-		9	-		0.5	-		9	±		9	-		9	+		9	±		20+	+		5	-		20++	-		20+	-		20+	-		20+	-	
		20+	-	.020	20+	-	.020	0.5	-	NA	5	-	NA	20++	++	.140	0.5	-	NA	20++	+	.060	20+	-	.020	20++	++	.140	20++	+	.060	20++	+++	.110	20+	-	.020	20++	-	alk	20++	-	.030	20++	-	alk	20++	-	NA
	72	20+	-	.020	20+	-	.020	0.5	-	NA	5	-	NA	20++	++	.135	0.5	-	NA	20++	+	.070	20+	-	.020	20++	++	.115	20++	++	.070	20++	+++	.110	20+	±	.020	20++	-	alk	20++	-	.025	20++	-	alk	20++	-	NA
		0.2	-		0.2	-		0.5	-		0.5	-		5	-		0.5	-		5	-		9	-		0.2	-		0.2	-		20+	-		0.2	-		0.5	-		0.2	-		20+	-		9	-	
	144	0.2	-		0.2	-		0.5	-		0.5	-		5	-		0.5	-		5	-		9	-		0.2	-		0.2	-		20+	-		0.2	-		0.5	-		0.2	-		20+	-		9	-	
		5	-		5	-		5	-		20+	-		20+	-		5	-		20+	-		20++	-		5	-		5	-		20++	-		5	-		5	-		0.5	-		20++	-		20+	-	
4°	192	5	-		5	-		5	-		20+	-		20+	-		5	-		20+	-		20++	-		5	-		5	-		20++	-		5	-		5	-		0.5	-		20++	-		20+	-	
		20+	-	.010	20+	-	.020	20++	-	alk	20+	-	alk	20++	-	.030	20++	-	NA	20++	-	.030	20++	++	.050	20+	+	.040	20+	-	NA	20++	++	.060	20++	-	.030	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA
	240	20+	-	.020	20+	-	.020	20++	-	alk	20+	-	alk	20++	±	.035	20++	-	NA	20++	-	.030	20++	++	.040	20+	+	.035	20+	-	NA	20++	++	.060	20++	-	.030	20+	-	alk	20+	-	alk	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 14 The fermentative ability of strain p182 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	-			-			-			-			0.05	-		-			0.2	-		0.05	-	
		-			-			-			-			0.05	-		-			0.2	-		0.05	-	
	48	-			-			-			-			0.1	- alk		-			0.5	-	NA	0.1	- alk	
		-			-			-			-			0.1	- alk		-			0.5	-	NA	0.1	- alk	
25°	24	5	-		5	-		0.5	-		0.5	-		9	-		0.5	-		9	-		20++	-	
		5	-		5	-		0.5	-		0.5	-		9	-		0.5	-		9	-		20++	-	
	48	9	-	.010	20+	-	.020	20+	+	.090	20+	++	.065	20+	- alk		20+	+	.045	20++	-	.025	20++	-	.020
		9	-	.010	20+	-	.020	20+	+	.090	20+	++	.070	20+	- alk		20+	+	.050	20++	-	.025	20++	-	.020
15°	24	0.2	-		0.2	-		0.1	-		0.1	-		5	-		0.1	-		5	-		0.2	-	
		0.2	-		0.2	-		0.1	-		0.1	-		5	-		0.1	-		5	-		0.2	-	
	48	5	-		9	-		5	-		9	-		20++	+		9	-		20++	+		20+	-	
		5	-		9	-		5	-		9	-		20++	+		9	-		20++	+		20+	-	
	72	20++	-	.020	9	-	.025	20++	-	.055	20++	+	.060	20++	++	.105	20++	-	.035	20++	++	.045	20++	-	.025
		20++	-	.020	9	-	.020	20++	-	.060	20++	+	.060	20++	++	.115	20++	-	.040	20++	++	.050	20++	-	.025
4°	144	0.2	-		0.2	-		5	-		5	-		0.5	-		5	-		0.5	-		0.5	-	
		0.2	-		0.2	-		5	-		5	-		0.5	-		5	-		0.5	-		0.5	-	
	192	9	-		9	-		20+	-		20+	-		5	-		20++	-		9	-		20+	-	
		9	-		9	-		20+	-		20+	-		5	-		20++	-		9	-		20+	-	
	240	20+	-	.020	20+	-	.020	20++	-	.030	20++	-	.020	20+	- alk		20++	-	.020	20++	-	NA	20++	- alk	
		20+	-	.020	20+	-	.015	20++	-	.030	20++	-	.020	20+	- alk		20++	-	.105	20++	-	NA	20++	- alk	

incubation temp.	incubation hours	lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	-		0.2	-		0.2	-		0.1	±		-			-			0.5	±+		0.1	-	
		0.1	±		0.2	±		0.1	-		-	±		-			-			0.5	±+		0.1	-	
	48	0.5	+	.055	0.5	+	.045	0.2	-	NA	0.2	+	.025	0.1	-	NA	0.1	-	.010	0.5	±	NA	0.2	- alk	
		0.5	+	.065	0.5	+	.045	0.2	-	NA	0.2	+	.030	0.05	-	NA	0.1	-	.020	0.5	±	NA	0.2	- alk	
25°	24	20+	-		20+	-		20+	+		5	++		9	-		20+	-		9	++		5	++	
		20+	-		20+	-		20+	+		5	++		9	-		20+	-		9	++		5	++	
	48	20++	- alk		20++	- alk		20++	++	.090	20++	++	.060	20++	-	NA	20++	-	.015	20++	+++	.130	20++	+++	.065
		20++	- alk		20++	- alk		20++	++	.080	20++	++	.060	20++	-	NA	20++	-	.015	20++	+++	.125	20++	+++	.070
15°	24	5	-		5	-		0.5	-		0.2	-		0.5	-		0.5	-		0.5	-		0.5	-	
		5	-		5	-		0.5	-		0.2	-		0.5	-		0.5	-		0.5	-		0.5	-	
	48	20++	+		20++	+		20+	-		5	-		20++	-		20+	-		20+	-		20+	-	
		20++	+		20++	+		20+	-		5	-		20++	-		20+	-		20+	-		20+	-	
	72	20++	++	.105	20++	++	.045	20++	+++	.110	20++	-	.020	20++	- alk		20++	-	.035	20++	-	NA	20++	-	NA
		20++	++	.115	20++	++	.050	20++	+++	.110	20++	-	.020	20++	- alk		20++	-	.035	20++	-	NA	20++	-	NA
4°	144	0.5	-		0.2	-		20++	-		0.2	-		0.5	-		0.5	-		20+	-		20+	-	
		0.5	-		0.2	-		20++	-		0.2	-		0.5	-		0.5	-		20+	-		20+	-	
	192	9	-		5	-		20++	-		5	-		5	-		5	-		20++	-		20++	-	
		9	-		5	-		20++	-		5	-		5	-		5	-		20++	-		20++	-	
	240	20++	-	.020	20+	-	NA	20++	-	.045	20+	+	.035	9	- alk		20+	- alk		20++	- alk		20++	-	NA
		20++	±	.030	20+	-	NA	20++	-	.040	20+	+	.035	9	- alk		20+	- alk		20++	- alk		20++	-	NA

Abbreviation and symbols refer Table 1

Table 15 The fermentative ability of strain 218 on several carbohydrates at different temperatures

incubation temp.	incubation hours	glucose			galactose			fructose			mannitol			rhamnose			arabinose			xylose			sorbitol			lactose			maltose			sucrose			raffinose			inulin			starch			lactic acid			glycerol		
		growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value	growth	gas	tit value
37°C	24	0.1	±		0.1	-		-			-			0.2	+		-			0.5	-		0.5	+		?			0.1	-		0.2	-		0.05	-		-			-			0.1	-		0.5	-	
		0.1	±		0.1	-		-			-			0.2	+		-			0.5	-		0.5	+		0.05	-		0.1	-		0.2	-		?			-			-			0.1	-		0.5	-	
	48	0.5	++	.065	0.5	++	.050	-			-			0.2	+	.055	-			5	-	.035	20+	++	.045	0.1	-	NA	0.2	-	NA	5	+	.080	0.1	-	NA	-			-			0.2	-	.015	5	-	.045
		0.5	++	.065	0.5	++	.060	-			-			0.2	+	.060	-			5	-	.035	20+	++	.045	0.1	-	NA	0.2	-	NA	5	+	.070	0.1	-	NA	-			-			0.2	-	.010	5	-	.040
25°	24	0.5	+		5	+		0.05	-		0.1	-		20+	+		-			9	++		20+	+		5	-		0.5	-		20+	-		0.1	-		9	-		9	-		0.5	-		0.5	-	
		0.5	+		5	+		0.1	+		0.1	-		20+	+		-			9	++		20+	+		5	-		0.5	-		20+	±		0.1	-		9	-		9	-		0.5	-		0.5	-	
	48	20+	++	.080	20+	++	.110	20+	++	.090	20+	+	.055	20++	++	.125	20+	+	.055	20++	+++	.055	20++	+++	.130	20++	-	alk	20++	+++	.030	20++	+++	.110	0.5	-	alk	20++	-	alk	20++	-	alk	9	-	alk	9	-	alk
		20+	++	.080	20+	++	.090	20+	++	.090	20+	+	.055	20++	++	.115	20+	+	.065	20++	+++	.060	20++	+++	.125	20++	-	alk	20++	+++	.030	20++	+++	.105	0.5	-	alk	20++	-	alk	20++	-	alk	9	-	alk	9	-	alk
	24	-			-			-			-			0.2	-		-			0.5	-		-			0.2	-		0.5	-		-			-			0.2	-		0.5	-		-			-		
		-			-			-			-			0.2	-		-			0.5	-		-			0.2	-		0.5	-		-			-			0.2	-		0.5	-		-			-		
15°	48	5	+		9	+		0.2	-		0.5	-		0.5	-		0.5	-		5	-		9	+		0.5	-		5	-		9	-		9	+		20+	-		20++	-		9	-		0.5	-	
		5	+		9	+		0.2	-		0.5	-		0.5	-		0.5	-		5	-		9	+		0.5	-		5	-		9	-		9	+		20+	-		20++	-		9	-		0.5	-	
	72	20+	++	.060	20++	++	.070	20+	+	.050	20++	-	.050	5	-	alk	5	-	.030	9	-	alk	20++	++	.070	5	-	alk	9	-	alk	20++	+	.100	20+	++	.060	20++	-	alk	20++	-	alk	20++	-	NA	20++	++	.035
		20+	++	.060	20++	++	.070	20+	+	.060	20++	-	.050	5	-	alk	5	-	.040	9	-	alk	20++	++	.060	5	-	alk	9	-	alk	20++	+	.090	20+	++	.050	20++	-	alk	20++	-	alk	20++	-	NA	20++	++	.035
	144	0.2	-		0.5	-		5	-		5	-		0.5	-		9	-		0.5	-		0.5	-		0.2	-		0.2	-		9	-		0.2	-		0.5	-		0.5	-		5	-		0.5	-	
		0.2	-		0.5	-		5	-		5	-		0.5	-		9	-		0.5	-		0.5	-		0.2	-		0.2	-		9	-		0.2	-		0.5	-		0.5	-		5	-		0.5	-	
4°	192	5	-		5	-		20+	-		20+	-		9	-		20+	-		9	-		9	-		0.2	-		0.5	-		20++	-		0.2	-		9	-		5	-		20+	-		20+	-	
		5	-		5	-		20+	-		20+	-		9	-		20+	-		9	-		9	-		0.2	-		0.5	-		20++	-		0.2	-		9	-		5	-		20+	-		20+	-	
	240	20+	±	.040	20+	-	.040	20++	-	.020	20+	-	NA	20++	-	.030	20++	-	.050	20++	-	NA	20++	-	NA	0.5	-	NA	5	-	NA	20++	-	.040	0.5	-	NA	20+	-	alk	20++	-	alk	20++	-	alk	20++	-	NA
		20+	±	.050	20++	-	.040	20++	-	.020	20+	-	NA	20++	-	.025	20++	-	.045	20++	-	NA	20++	-	NA	0.5	-	NA	5	-	NA	20++	-	.045	0.5	-	NA	20+	-	alk	20++	-	alk	20++	-	alk	20++	-	NA

Abbreviation and symbols refer Table 1

Table 16 is a summary of the effect of different incubation temperatures on the fermentative ability of fifteen strains of psychrophiles in different carbohydrate media. One strain, S12, gave similar carbohydrate fermentation reactions at different incubation temperatures whereas the rest of the strains showed different fermentation reactions at different incubation temperatures. The percentage of strains producing gas at 37°, 25°, 15° and 4°C incubated was respectively 20.9, 36.0, 31.4, 11.7. Of the strains fermenting at 15°C, over 62% failed to ferment the carbohydrates at 4°C.

Table 16 The effect of temperature on the fermentative ability of fifteen psychrophilic strains in different carbohydrate media

carbohydrate	strains #1	#2	S6	S12	p5	p7	p8	p9	p13	p15[I]	p19	p152	p181	p182	218
glucose	- +	- +	+ +	+ +	+ +	- -	- -	- -	- -	- +	/ -	- -	- -	/ -	+ +
	- -	- -	+ -	+ +	+ +	- -	- -	- -	- -	- -	- -	- -	- -	- -	+ +
galactose	- -	- -	+ +	+ +	+ +	- -	+ +	- -	- -	- -	/ -	- -	- -	/ -	+ +
	- -	- -	+ -	+ +	+ +	- -	+ +	- -	- -	- -	- -	- -	- -	- -	+ -
fructose	- +	/ +	/ +	/ +	/ +	/ -	/ -	/ +	/ -	/ -	/ +	/ +	/ -	/ -	/ +
	+ -	+ -	+ +	+ +	+ +	- -	+ -	+ -	+ -	- -	+ -	+ -	- -	- -	+ -
mannitol	/ +	/ +	/ +	/ +	/ +	/ -	/ +	/ +	/ -	/ -	/ +	/ +	/ -	/ +	/ -
	+ -	+ -	+ -	+ +	+ +	- -	+ -	+ -	+ -	+ -	+ -	+ -	- -	+ -	+ -
rhamnose	- -	+ +	+ +	+ +	+ +	- +	- -	+ +	+ +	+ +	- -	- -	+ +	- +	+ -
	- -	+ +	+ +	+ +	+ +	+ -	- -	+ -	+ -	+ -	- -	- -	+ +	- -	+ -
arabinose	/ -	/ +	/ -	/ +	/ +	/ -	/ -	/ -	/ -	/ -	/ -	/ -	/ -	/ -	/ -
	+ -	+ -	+ -	+ +	+ +	- -	- -	+ -	+ -	- -	+ -	+ -	- -	+ -	+ -
xylose	/ -	+ +	+ +	+ +	+ +	+ +	- -	+ +	+ +	+ +	+ -	- -	+ +	- +	+ -
	- -	+ +	+ -	+ +	+ +	+ -	- -	+ -	+ -	+ -	+ -	- -	+ -	- -	+ -
sorbitol	- -	+ +	+ +	+ +	+ +	+ +	- -	+ +	+ +	+ +	- -	- -	+ +	- -	+ +
	- -	+ +	+ +	+ +	+ +	+ -	- -	+ -	+ -	+ -	- -	- -	+ +	- -	+ -
lactose	- -	/ -	/ -	+ +	+ +	- -	+ +	- -	- -	+ +	- -	- -	+ +	+ +	- -
	- -	- -	- -	+ +	+ +	- -	+ -	- -	- -	+ -	- -	- -	+ +	+ -	- -
maltose	/ -	/ -	/ -	+ +	+ +	- -	+ +	- -	- -	+ +	- -	/ -	+ +	+ +	- -
	- -	- -	- -	+ +	+ +	- -	+ +	- -	- -	+ -	- -	- -	+ -	- -	+ -
sucrose	+ -	- -	- +	+ +	+ +	- +	- +	- -	- +	- -	- +	+ +	- +	- +	+ +
	+ -	- -	+ -	+ +	+ -	+ -	+ +	+ -	+ -	- -	+ +	+ -	+ +	+ -	+ -
raffinose	/ -	/ -	/ -	+ +	+ +	- -	+ +	- -	- -	+ +	- -	- -	+ -	+ +	/ +
	- -	- -	- -	+ +	+ -	- -	+ -	- /	- -	+ -	- -	- -	+ -	+ +	- -
inulin	/ -	/ -	/ -	- -	- +	- -	- -	- -	- -	- -	/ +	+ +	- -	- -	/ -
	- -	- -	- -	- -	+ -	- -	- -	- -	- -	- -	+ -	+ -	- -	- -	- -
starch	/ -	- -	/ -	- -	- +	- -	- -	- -	- -	- -	- -	- +	- -	- -	- -
	- -	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	+ -	- -	- -	- -
lactic acid	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	+ -	- -
	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	+ -	- -
glycerol	+ +	- -	- -	- -	+ +	- -	- -	- -	- -	- -	- -	+ +	- -	- -	- +
	+ -	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	+ -	- -	+ -	- -

37° 15°
25° 4°

In each square the incubation temperature used is located as, 37°C - upper left corner, 25°C - lower left corner, 15°C - upper right corner and 4°C - lower right corner, and the degree of growth and degree of gas production are not shown.

/, no observation; -, no gas production; +, gas production

Tables 17 - 31 show the reaction in litmus milk by fifteen strains of psychrophiles incubated at different temperatures. At 37°C, almost all the strains had little effect on pH, reduction of the dye, coagulation or peptonization, though some produced slight acid. At 25°C, almost half of the strains tested produced strong reduction, coagulation and peptonization. Five out of six strains which were inert at 37°C gave an alkaline reaction though the number of acid producers remained the same. At 15°C, the degree of reduction coagulation and peptonization decreased a little, in comparison with that at 25°C; there was no particular change in the proportion of each type of reaction. At 4°C, the number of acid producere was les and the number of inert and indistinct reactions correspondingly greater.

One strain produced an acid reaction at both 37° and 25°C but gave an alkaline reaction at 4°C. Apart from this strain, all others gave a narrower range of reaction at different temperatures and periods of incubation: e.g. inert > acid, or inert > alkali, or vice versa but not acid > alkali or vice versa.

Table 17 The effect of strain #1 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag*1	peptonization
		no change	-*2	-	-
	24	no change	-	-	-
		no change	-	-	-
37°C	48	no change	-	-	-
		sl. acid*3	-	-	-
	72	no change	-	-	-
		acid	+	-	-
	24	acid	+	-	-
		acid	+++	+++	-
25°	48	acid	+++	+++	-
		acid	+++	+++	±
	72	acid	+++	+++	±
		acid	+	-	±
	24	acid	+	-	±
		acid	++	-	±
15°	48	acid	++	-	±
		acid	++	+++	±
	72	acid	++	+++	±
		acid	+	-	-
	144	acid	+	-	-
		acid	++	-	-
4°	192	acid	++	-	-
		acid	+++	-	-
	240	acid	+++	-	-

*1 coag: coagulation
*2 -, negative; ±, doubtful; +, slight; ++, moderate +++, strong
*3 sl. acid - slightly acid

Table 18 The effect of strain #2 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
37°C	48	no change	-	-	-
		no change	-	-	-
	72	no change	-	-	-
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
25°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	24	no change	-	-	-
		alkali	-	-	-
15°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	144	no change	-	-	-
		alkali	-	-	-
4°	192	alkali	-	-	-
		alkali	-	-	-
	240	alkali	-	-	-

Symbols and abbreviation refer Table 17

Table 19 The effect of strain S6 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
37°C	48	no change	-	-	-
		no change	-	-	-
	72	no change	-	-	-
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
25°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		sl. alkali*1	-	-	-
	24	no change	-	-	-
		alkali	-	-	-
15°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		sl. alkali	-	-	-
	144	sl. alkali	-	-	-
		alkali	-	-	-
4°	192	alkali	-	-	-
		alkali	-	-	-
	240	alkali	-	-	-

Symbols and abbreviation refer Table 17

*1 sl. alkali - slightly alkali

Table 20 The effect of strain S12 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		acid	-	-	-
	24	acid	-	-	-
		acid	-	-	-
37°C	48	acid	-	-	-
		acid	-	+++	±
	72	acid	-	+++	-
		acid	-	±	+
	24	acid	-	+	+
		acid	++	+++	+++
25°	48	acid	+++	+++	+++
		acid	+++	+++	+++
	72	acid	+++	+++	+++
		acid	-	-	±
	24	acid	-	-	±
		acid	-	-	±
15°	48	acid	-	±	±
		acid	++	+	+++
	72	acid	++	+	+++
		sl. acid	±	-	+
	144	sl. acid	±	-	+
		?*1	+	-	++
4°	192	?	+	-	++
		?	+	-	+++
	240	?	+	-	+++

Symbols and abbreviation refer Table 17

*1 acid or alkali was not measured because of high reduction and/or peptonization

Table 21 The effect of strain p5 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		acid	-	-	-
	24	acid	-	-	-
		acid	-	-	-
37°C	48	acid	-	-	-
		acid	-	-	-
	72	acid	-	-	-
		acid	-	-	-
	24	acid	-	-	-
		acid	+	++	++
25°	48	acid	+	++	++
		acid	++	+++	+++
	72	acid	++	+++	+++
		acid	-	-	-
	24	acid	-	-	-
		acid	-	-	-
15°	48	acid	-	-	-
		acid	++	++	+++
	72	acid	++	++	+++
		no change	-	-	-
	144	no change	-	-	-
		acid	-	-	-
4°	192	acid	-	-	-
		acid	±	-	++
	240	acid	±	-	++

Symbols and abbreviation refer Table 17

Table 22 The effect of strain p7 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		alkali	-	-	-
	24	alkali	-	-	-
		no change	-	-	-
37°C	48	no change	-	-	-
		no change	-	-	-
	72	no change	-	-	-
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
25°	48	no change	-	-	-
		no change	-	-	-
	72	no change	-	-	-
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
15°	48	no change	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	144	no change	-	-	-
		no change	-	-	-
4°	192	no change	-	-	-
		no change	-	-	-
	240	no change	-	-	-

Symbols and abbreviation refer Table 17

Table 23 The effect of strain p8 on litmus milk at different temperatures.

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		acid	-	-	-
	24	acid	-	-	-
		acid	-	-	-
37°C	48	acid	-	-	-
		acid	-	-	-
	72	acid	-	-	-
		acid	-	-	-
	24	acid	-	-	-
		acid	+	++	+++
25°	48	acid	+	++	+++
		acid	++	+++	+++
	72	acid	++	+++	+++
		alkali	-	-	-
	24	alkali	-	-	-
		no change	±	-	-
15°	48	no change	±	-	-
		acid	++	++	+++
	72	acid	++	++	+++
		no change	-	-	-
	144	no change	-	-	-
		sl. alkali	-	-	-
4°	192	sl. alkali	-	-	-
		alkali	-	-	+++
	240	alkali	-	-	+++

Symbols and abbreviation refer Table 17

Table 24 The effect of strain p9 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
37°C	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
25°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
15°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	144	no change	-	-	-
		no change	-	-	-
4°	192	no change	-	-	-
		no change	-	-	-
	240	no change	-	-	-

Symbols and abbreviation refer Table 17

Table 25 The effect of strain p13 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		alkali	-	-	-
	24	alkali	-	-	-
		no change	-	-	-
37°C	48	no change	-	-	-
		sl. acid	-	-	-
	72	sl. acid	-	-	-
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
25°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
15°	48	no change	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		sl. alkali	-	-	-
	144	sl. alkali	-	-	-
		alkali	-	-	-
4°	192	alkali	-	-	-
		alkali	-	-	-
	240	alkali	-	-	-

Symbols and abbreviation refer Table 17

Table 26 The effect of strain p15[I] on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		sl. acid	-	-	-
	24	sl. acid	-	-	-
		acid	-	-	-
37°C	48	acid	-	-	-
		acid	-	+	-
	72	acid	-	+	-
		acid	-	-	-
	24	acid	-	-	-
		acid	+	+	++
25°	48	acid	+	+	++
		acid	++	+++	+++
	72	acid	++	+++	+++
		acid	-	-	-
	24	acid	-	-	-
		acid	+	-	-
15°	48	acid	±	-	-
		acid	+	-	++
	72	acid	+	-	++
		sl. acid	-	-	-
	144	sl. acid	-	-	-
		acid	-	-	±
4°	192	acid	-	-	±
		acid	+	-	+++
	240	acid	+	-	+++

Symbols and abbreviation refer Table 17

Table 27 The effect of strain p19 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
37°C	48	alkali	-	-	-
		no change	-	-	-
	72	no change	-	-	-
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
25°	48	no change	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
15°	48	no change	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	144	no change	-	-	-
		no change	-	-	-
4°	192	no change	-	-	-
		no change	-	-	-
	240	no change	-	-	-

Symbols and abbreviation refer Table 17

Table 28 The effect of strain p152 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
37°C	48	alkali	-	-	-
		no change	-	-	-
	72	no change	-	-	-
		no change	-	-	-
	24	no change	-	-	-
		no change	-	-	-
25°	48	no change	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
15°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	144	no change	-	-	-
		no change	-	-	-
4°	192	no change	-	-	-
		no change	-	-	-
	240	no change	-	-	-

Symbols and abbreviation refer Table 17

Table 29 The effect of strain p181 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		acid	-	-	-
	24	acid	-	-	-
		acid	-	-	-
37°C	48	acid	-	-	-
		acid	-	±	-
	72	acid	-	-	-
		acid	-	-	+
	24	acid	-	-	+
		acid	+	+	++
25°	48	acid	+	+	++
		acid	++	+++	+++
	72	acid	++	+++	+++
		no change	-	-	-
	24	no change	-	-	-
		acid	-	-	-
15°	48	acid	-	-	-
		acid	±	-	±
	72	acid	+	-	+
		no change	-	-	-
	144	no change	-	-	-
		?	-	-	+
4°	192	?	-	-	+
		?	+	-	+++
		?	+	-	+++

Symbols and abbreviation refer Table 17

Table 30 The effect of strain p182 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		acid	-	-	-
	24	acid	-	-	-
		acid	-	-	-
37°C	48	acid	-	-	-
		acid	-	-	-
	72	acid	-	-	-
		acid	-	-	+
	24	acid	-	-	+
		acid	+	+	+++
25°	48	acid	+	+	+++
		acid	++	+++	+++
	72	acid	++	+++	+++
		no change	-	-	-
	24	no change	-	-	-
		acid	+	-	-
15°	48	acid	+	-	-
		acid	++	+	+++
	72	acid	++	+	+++
		sl. acid	-	-	-
	144	sl. acid	-	-	-
		?	±	-	+
4°	192	?	±	-	+
		?	+	-	+++
	240	?	+	-	+++

Symbols and abbreviation refer Table 17

Table 31 The effect of strain 218 on litmus milk at different temperatures

incubation temp.	incubation hours	alkali acid	reduction	coag	peptonization
		no change	-	-	-
	24	no change	-	-	-
		alkali	-	-	-
37°C	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		alkali	-	-	-
	24	alkali	-	-	-
		alkali	-	-	-
25°	48	alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		sl. alkali	-	-	-
	24	sl. alkali	-	-	-
		sl. alkali	-	-	-
15°	48	sl. alkali	-	-	-
		alkali	-	-	-
	72	alkali	-	-	-
		no change	-	-	-
	144	no change	-	-	-
		no change	-	-	-
4°	192	no change	-	-	-
		alkali	-	-	-
	240	alkali	-	-	-

Symbols and abbreviation refer Table 17

Table 32 is a summary of the effect of different incubation temperatures on the litmus milk reaction of fifteen strains of psychrophiles.

If the optimum temperature for psychrophiles were between 25° and 15°C, then it would be expected that the maximum reaction would be noted at these temperatures.

There is insufficient data in Table 32 to be able to say that the cultures are representative of psychrophiles in general but the values are not out of keeping with such an idea.

Table 32 The effect of temperature on reaction in litmus milk with 15 strains of psychrophiles

degree of reaction	Reaction of at °C											
	reduction				coagulation				peptonization			
	37°	25°	15°	4°	37°	25°	15°	4°	37°	25°	15°	4°
-	15	8	8	9	12	8	10	15	14	8	8	9
±				1	1				1	1		
+			2	4	1		2				1	
++		5	5				2				1	1
+++		2		1	1	7	1			6	3	5

Tables 33 - 47 show the nitrate reduction by fifteen strains of psychrophiles at different temperatures. Few of the strains brought about any reduction of nitrate. However with those that did there is evidence of differences in nitrate reduction at different temperatures.

Table 39 shows a strain which produced N_2 gas at 37°C without apparent reduction of nitrate. Another strain gives the same reaction in Table 37 at both 37° and 25°C. Yet another strain in Table 36 shows the phenomenon at 25°C.

One possible explanation might be that ammonia was liberated from protein in the medium by these strains. Warren et al. (1960) postulated that resting cells of Ps. aeruginosa in the presence of an oxidizable substance such as glucose, had the general economy of the cells changed so that they could reincorporate an end-product of endogenous respiration, namely ammonia.

Table 33 The ability of strain #1 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth*1	N_2gas*2	reduction*3
		0.1	-	
	24	0.1	-	
37°C				
		0.1	-	-
	48	0.1	-	-
		9	-	
	24	9	-	
25°				
		9	-	-
	48	9	-	-
		5	-	
	24	5	-	
		9	-	
15°	48	9	-	
		9	-	-
	72	9	-	-
		0.5	-	
	144	0.5	-	
		9	-	
4°	192	9	-	
		20+	-	-
	240	20+	-	-

*1 growth: O.D. x 10 (the higher reading taken)

*2 N_2 gas: -, negative; ±, very little; +, slight; ++, good; +++, abundant

*3 reduction: -, no reduction; +, reduction

Table 34 The ability of strain #2 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.05	-	
	24	0.05	-	
37°C				
		0.1	-	-
	48	0.1	-	-
		5	-	
	24	5	-	
25°				
		5	-	-
	48	5	-	-
		5	-	
	24	5	-	
		9	-	
15°	48	9	-	
		9	-	-
	72	9	-	-
		5	-	
	144	5	-	
		5	-	
4°	192	5	-	
		20+	-	-
	240	20+	-	-

Symbols and abbreviation as in Table 33

Table 35 The ability of strain S6 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.2	-	
	24	0.2	-	
37°C				
		0.2	-	-
	48	0.2	-	-
		9	-	
	24	9	-	
25°				
		20+	-	-
	48	20+	-	-
		5	-	
	24	5	-	
		9	-	
15°	48	9	-	
		9	-	-
	72	9	-	-
		5	-	
	144	5	-	
		9	-	
4°	192	9	-	
		20+	-	-
	240	20+	-	-

Symbols and abbreviation as in Table 33

Table 36 The ability of strain S12 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.2	-	
	24	0.2	-	
37°C				
		0.2	-	-
	48	0.2	-	-
		5	-	
	24	5	-	
25°				
		20+	+	-
	48	20+	+	-
		5	-	
	24	5	-	
		20+	++	
15°	48	20+	++	
		20+	+++	+
	72	20+	+++	+
		5	+	
	144	5	+	
		5	++	
4°	192	5	++	
		20+	+++	+
	240	20+	+++	+

Symbols and abbreviation as in Table 33

Table 37 The ability of strain p5 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.5	+	
	24	0.5	+	
37°C				
		0.5	+	-
	48	0.5	+	-
		0.5	±	
	24	0.5	+	
25°				
		20+	±	-
	48	20+	+	-
		5	-	
	24	5	-	
		9	++	
15°	48	9	++	
		9	+++	+
	72	9	+++	+
		0.5	-	
	144	0.5	-	
		5	±	
4°	192	5	±	
		9	+	+
	240	9	+	+

Symbols and abbreviation as in Table 33

Table 38 The ability of strain p7 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.2	-	
	24	0.2	-	
37°C				
		0.5	-	-
	48	0.5	-	-
		0.2	-	
	24	0.2	-	
25°				
		5	-	-
	48	5	-	-
		0.2	-	
	24	0.2	-	
		0.5	-	
15°	48	0.5	-	
		5	-	-
	72	5	-	-
		0.1	-	
	144	0.1	-	
		0.1	-	
4°	192	0.1	-	
		0.2	-	-
	240	0.2	-	-

Symbols and abbreviation as in Table 33

Table 39 The ability of strain p8 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.05	±	
	24	0.05	±	
37° C				
		0.1	+	-
	48	0.1	+	-
		0.5	-	
	24	0.5	-	
25°				
		0.5	-	-
	48	0.5	-	-
		0.1	-	
	24	0.1	-	
		0.2	-	
15°	48	0.2	-	
		0.5	-	-
	72	0.5	-	-
		0.1	-	
	144	0.1	-	
		0.1	-	
4°	192	0.1	-	
		0.1	-	-
	240	0.1	-	-

Symbols and abbreviation as in Table 33

Table 40 The ability of strain p9 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.05	-	
	24	0.05	-	
37° C				
		0.1	±	-
	48	0.1	±	-
		5	-	
	24	5	-	
25°				
		5	-	-
	48	5	-	-
		0.2	-	
	24	0.2	-	
		5	-	
15°	48	5	-	
		5	-	-
	72	5	-	-
		0.1	-	
	144	0.1	-	
		0.1	-	
4°	192	0.1	-	
		0.1	-	-
	240	0.1	-	-

Symbols and abbreviation as in Table 33

Table 41 The ability of strain p13 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.1	-	
	24	0.1	-	
37°C				
		0.1	-	-
	48	0.1	-	-
		0.2	-	
	24	0.2	-	
25°				
		0.5	-	-
	48	0.5	-	-
		5	-	
	24	5	-	
		9	-	
15°	48	9	-	
		9	-	-
	72	9	-	-
		5	-	
	144	5	-	
		9	-	
4°	192	9	-	
		20+	-	-
	240	20+	-	-

Symbols and abbreviation as in Table 33

Table 42 The ability of strain p15[I] to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.2	-	
	24	0.2	-	
37°C				
		0.2	-	-
	48	0.2	-	-
		0.2	-	
	24	0.2	-	
25°				
		5	-	-
	48	5	-	-
		0.2	-	
	24	0.2	-	
		0.5	-	
15°	48	0.5	-	
		5	-	-
	72	5	-	-
		0.1	-	
	144	0.1	-	
		0.2	-	
4°	192	0.2	-	
		0.2	-	+
	240	0.2	-	+

Symbols and abbreviation as in Table 33

Table 43 The ability of strain p19 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.1	-	
	24	0.1	-	
37°C				
		0.1	-	-
	48	0.1	-	-
		0.1	-	
	24	0.1	-	
25°				
		0.2	-	-
	48	0.2	-	-
		0.05	-	
	24	0.05	-	
		0.1	-	
15°	48	0.1	-	
		0.2	-	-
	72	0.2	-	-
		?		
	144	?		
		?		
4°	192	?		
		0.05	-	-
	240	0.05	-	-

Symbols and abbreviation as in Table 33

Table 44 The ability of strain p152 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
37°C	24	0.2	-	
		0.2	-	
	48	0.2	-	-
		0.2	-	-
25°	24	5	-	
		5	-	
	48	9	-	-
		9	-	-
15°	24	0.2	-	
		0.2	-	
	48	5	-	
		5	-	
	72	5	-	-
		5	-	-
4°	144	0.1	-	
		0.1	-	
	192	0.1	-	
		0.1	-	
	240	0.2	-	-
		0.2	-	-

Symbols and abbreviation as in Table 33

Table 45 The ability of strain p181 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		0.1	-	
	24	0.1	-	
37°C				
		0.1	-	-
	48	0.1	-	-
		5	-	
	24	5	-	
25°				
		5	-	-
	48	5	-	-
		0.2	-	
	24	0.2	-	
		9	-	
15°	48	9	-	
		9	-	-
	72	9	-	-
		9	-	
	144	9	-	
		9	-	
4°	192	9	-	
		20+	-	-
	240	20+	-	-

Symbols and abbreviation as in Table 33

Table 46 The ability of strain p182 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
		?		
	24	?		
37°C				
		0.1	-	-
	48	0.1	-	-
		9	-	
	24	9	-	
25°				
		20+	-	+
	48	20+	-	+
		5	-	
	24	5	-	
		9	-	
15°	48	9	-	
		9	-	-
	72	9	-	-
		0.5	-	
	144	0.5	-	
		5	-	
4°	192	5	-	
		9	-	-
	240	9	-	-

Symbols and abbreviation as in Table 33

Table 47 The ability of strain 218 to reduce nitrate at different temperatures

incubation temp.	incubation hours	growth	N_2 gas	reduction
37°C	24	-		
		-		
	48	-		
		-		
25°	24	-		
		-		
	48	-		
		0.1	-	
	24	0.1	-	
		0.1	-	
15°	48	0.1	-	
		0.1	-	-
	72	0.1	-	-
		0.1	-	
	144	0.1	-	
		0.1	-	
4°	192	0.1	-	
		0.2	-	-
	240	0.2	-	-

Symbols and abbreviation as in Table 33

Table 48 shows the ability of fifteen strains of psychrophiles to liquefy gelatin at different temperatures. At 37°C, none showed an appreciable extent of liquefaction. At 25°C, four strains liquefied gelatin and while at 15°C there was a less extensive liquefaction the same strains were involved. At 4°C the pattern of liquefaction was similar. Prolonging the incubation to 30 days at 4°C allowed #1, S6, p5 and 218 to liquefy the gelatin whereas no appreciable change was observed for incubation at 37°, 25° and 15°C for 5, 7 and 7 days, respectively. Strains which produced doubtful readings at the above temperatures did not liquefy the gelatin any further. All the strains, except p9, grew well at 37°, 25° and 15° even after prolonged incubation whereas abundant growth was observed by incubation for 30 days at 4°C.

Table 4 8 Effect of temperature on gelatin liquefaction by fifteen strains of psychrophiles

incubation temp.	incubation hours	strain #	#1	#2	S6	S12	p5	p7	p8	p9	p13	p15[I]	p19	p
37°C	48		- *	-	-	-	-	-	-	-	-	-	-	
			-	-	-	-	-	-	-	-	-	-	-	
	72		-	-	-	-	-	-	-	-	-	±	-	
			-	-	-	-	-	-	-	-	-	±	-	
25°C	48		-	-	-	++	-	-	-	-	-	-	-	
			-	-	-	++	-	-	-	-	-	-	-	
	72		-	-	-	+++	-	-	-	-	++	-	-	
			-	-	-	+++	-	-	-	-	++	-	-	
15°C	48		-	-	-	+	-	-	-	-	-	-	-	
			-	-	-	+	±	-	-	-	-	-	-	
	72		-	-	-	+	±	-	-	-	+	-	-	
4°C	144		-	-	-	±	-	-	-	-	-	-	-	
			-	-	-	±	-	-	-	-	-	-	-	
	192		-	-	-	+	-	-	-	-	±	-	-	
			-	-	-	+	-	-	-	-	±	-	-	
	240		-	-	±	++	-	-	-					
			-	-	±	++	-	-	-	-	+	-	-	
										-	+	-	-	

* -, no liquefaction; ±, doubtful; +, a little; ++, moderately

+++, strongly

sychrophiles

3	p15[I]	p19	p152	p181	p182	218
	-	-	-	-	-	-
	-	-	-	-	-	-
	±	-	-	-	-	-
	±	-	-	-	-	-
	-	-	-	++	++	-
	-	-	-	++	++	-
	-	-	-	+++	++	-
	-	-	-	+++	++	-
	-	-	-	++	++	-
	-	-	-	++	++	-
	-	-	-	++	++	-
	-	-	-	++	++	-
	-	-	-	+	+	-
	-	-	-	+	+	-
	-	-	-	+	+	-
	-	-	-	+	+	-
	-	-	-	++	+++	-
	-	-	-	++	+++	-

Table 49 shows the results of morphological and microscopical observations, on growth on non-acidified Bacto-potato-dextrose agar. With regard to morphology, observations were made on pigment formation, hue of the pigment, surface of the colony, and edge of the colony. The terminology used to describe the above conditions was that proposed by Breed et al. (1957). Most strains produced white to very faint yellow non-diffusible pigment and a smooth surface. Microscopical features observed were shape of the cell, cell grouping, and the type of flagellation. The cells of most of the strains were short rods, occurring singly, in pairs, or in chains up to 6 cells.

None of the fifteen strains of psychrophiles showed appreciable differences in morphology or pigment formation at the different incubation temperatures.

Table 49 Morphology and microscopical features of fifteen strains of psychrophiles

strain

#1 short rods occurring singly, in pairs and in short chains up to 6 cells:
colonies - white to very faint yellow, opaque, circular, viscid, smooth

#2 short small rods occurring singly, in pairs and in chains:
colonies - white to faint yellow, opaque, circular, viscid, smooth

S6 short rods occurring singly, in pairs:
colonies - white to faint grey, opaque, circular, smooth, viscid

S12 rods occurring singly and in pairs:
colonies - white to grey, opaque, circular, smooth

p5 rods occurring singly, in pairs and in chains:
colonies - white to grey, opaque, circular, smooth

p7 round rods, occurring singly, in pairs:
colonies - white to very faint yellow, opaque, viscid, circular, smooth

p8 short small rods occurring singly and in pairs: lophotrich flagella (4 - 6)
colonies - spreading, white, viscid, undulate, sweet ester like odor.

p9 short small rods occurring most singly:
colonies - white to very faint yellow, opaque, viscid, circular, smooth

p13 short small rods occurring singly, in pairs and in chains:
colonies - white to yellow, greenish center, circular, viscid, smooth

p15[I] short small rods, occurring singly, in pairs and in chains
colonies - yellowish, opaque, viscid, circular, convex

p19 spheres, occurring in pairs:
colonies - white-dense to yellow, glistering, erose

p152 small round rods occurring mostly in pairs and clumps:
colonies - white to faint yellow, opaque, circular, smooth

p181 short small rods occurring mostly singly:
colonies - spreading, white, shiney, viscid, undulate, sweet ester like odor

p182 short rods occurring mostly singly:
colonies - spreading, white, shiney, viscid, undulate to erose, sweet ester odor

218 round rods, occurring singly, in pairs and in chains:
colonies - white to very faint yellow, opaque, auriculate to lacerate, viscid, smooth


undulate


erose


auriculate


lacerate

None of the fifteen strains of psychrophiles hydrolyzed starch at 37°C incubation whereas strain 218 hydrolyzed starch at 25°C and 15°C after incubation for 3 and 4 days, respectively, and strains p181 and 218 hydrolyzed starch at 4°C incubation after 10 days.

Only strain p5 produced indole at 37°, 25° and 15°C after 3 days incubation whereas no indole was detected at 4°C after incubation for 10 days.

Table 50 shows the tentative classification of the fifteen strains of psychrophiles, isolated from milk and milk products, and subjected to the above tests.

All the strains were catalase positive, and with the exception of p19, all the strains were Gram-negative.

Table 50 The tentative classification of the fifteen strains of psychrophiles

strain	classified as
#1	Pseudomonas taetrolens
#2	Alcaligenes viscolactis
S6	Achromobacter guttatus
S12	Pseudomonas putrefaciens
p5	Pseudomonas putrefaciens
p7	Alcaligenes viscolactis
p8	Pseudomonas fragi
p9	Alcaligenes viscolactis
p13	Pseudomonas fluorescens
$p15^{I}$	Pseudomonas taetrolens
p19	Micrococcus lutens
p152	Alcaligenes viscolactis
p181	Pseudomonas fragi
p182	Pseudomonas fragi
218	Alcaligenes viscolactis

II Determination of peptides

Table 51 and Graph 1 show the relationship between the concentration of a ninhydrin-positive amino group and the optical density at 580 mu of ninhydrin solution. An amino acid was spotted at various concentrations on Whatman #1 filter paper and chromatographed with n-butanol - glacial acetic acid - water (4 : 1 : 5) solvent. The spots, detected by spraying with 0.1% ninhydrin solution in 95% ethyl alcohol, were then eluted with 1 ml of distilled water and 3 ml of 0.4% ninhydrin solution in 95% ethyl alcohol in order to make color measurements on the spectrophotometer.

The concentration of a ninhydrin-positive amino group was calculated from the molecular weight of the amino acid used, and the optical density readings were calculated and adjusted to make a straight line, using linear regression analysis technique.

The individual differences between the observed and the calculated optical density values did not show any significance at the 50% point.

It was difficult to determine the concentration of color in ninhydrin solution by the spectrophotometer when the amino group was less than 0.3 μg; it was not possible to spot more than 1.0 ml of the dialysate which was condensed to a final volume of 5 ml because of its density.

Table 51 The relationship of observed and expected optical density at 580 mμ to concentration of amino group

μg NH_2 (/4 ml ninhydrin solution	optical density at 580mμ	
	observed	adjusted
0.48780	0.016	0.00969
0.60975	0.040	0.02398
0.73171	0.035	0.03827
0.85365	0.060	0.05256
0.97600	0.053	0.06685
1.21951	0.096	0.09543
1.46341	0.100	0.12401
1.82926	0.181	0.16687
2.43902	0.225	0.23823
3.04878	0.300	0.30978
3.65853	0.373	0.38121
4.26829	0.456	0.45266

calculation formula

$$Y = 0.11717\ x - 0.04746$$

where, Y, adjusted O.D.; x, μg amino group

Graph 1 The relationship of observed and expected optical density at 580 mμ to concentration of amino group

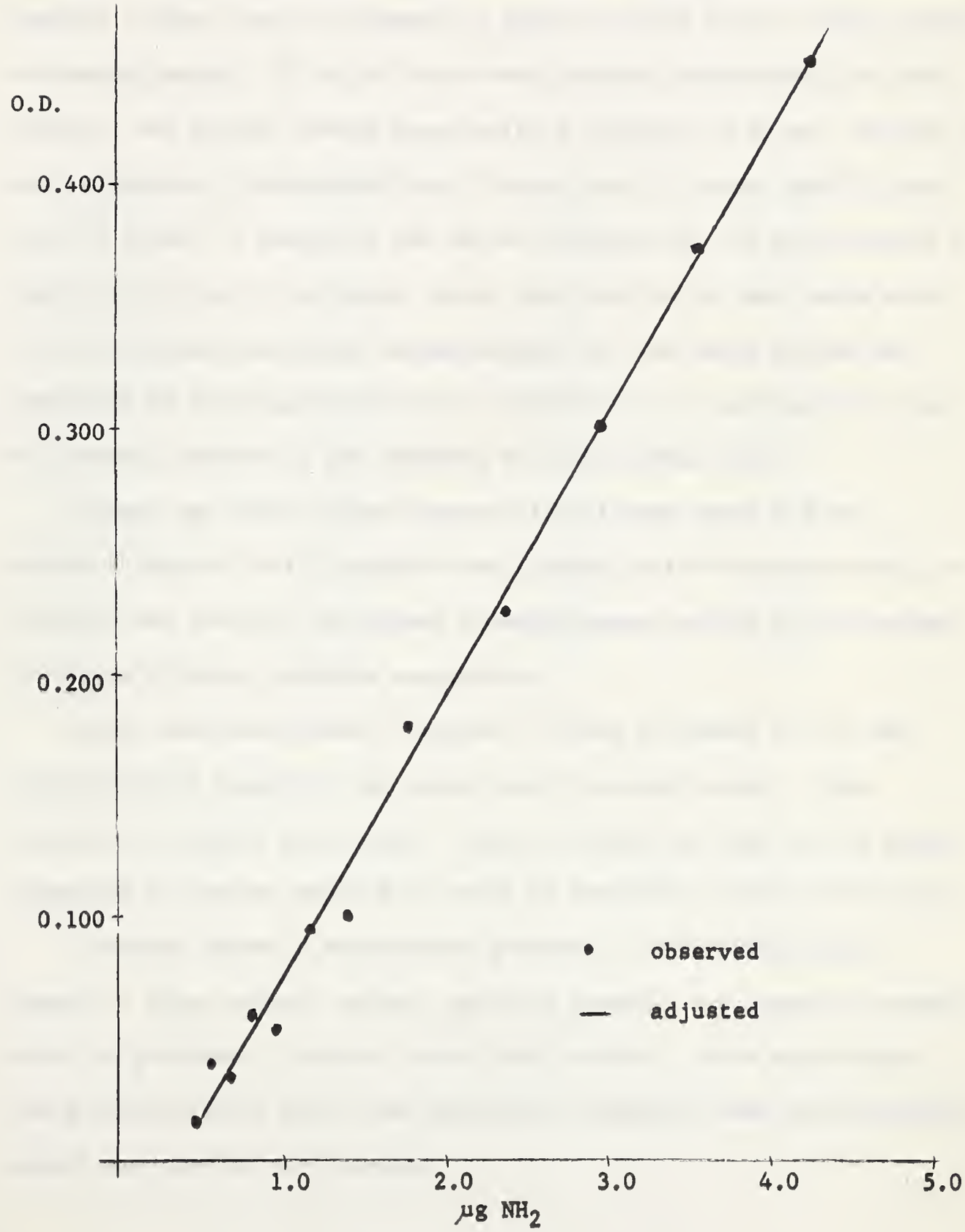


Table 52 shows the R_f and $R_{leucine}$ values of 19 commercially available natural L-amino acids on two-dimensional paper chromatography. Using the two following solvent systems, a) n-butanol - methylethylketone - ammonia - water, and b) n-butanol - glacial acetic acid - water, with the descending method, 72 and 23 hours were required respectively to move leucine, the fastest moving amino acid, a distance of 40 cm. In fact it was subsequently determined that 72 hours was 4.5 times, and 23 hours was 1.3 times, in excess of the period required for the each solvent to reach the bottom of the paper, hence the location of each amino acid with two-dimensional paper chromatography for the above system was expressed by dividing the distance travelled by an amino acid by that of leucine, instead of the distance of each solvent front.

Though the first solvent system alone listed above did not separate aspartic acid, cysteine and glutamic acid from each other, or tyrosine and proline, the second solvent system applied in the second dimension afforded adequate separation.

Water saturated phenol required 17 hours to travel 40 cm, and satisfactorily separated the amino acids from each other. When n-butanol - glacial acetic acid - water solvent was used in the second dimension an unknown amino acid could be identified without difficulty.

Tyrosine showed a streak with n-butanol - methylethylketone - ammonia - water solvent system, and both aspartic and tyrosine streaked with the n-butanol - glacial acetic acid solvent. Both tryptophane and proline did not give clear spots with ninhydrin when water saturated phenol was used as the solvent.

Table 52 R_f and $R_{leucine}$ values of L-amino acids

amino acid	solvent 1	solvent 2	solvent 3
	$R_{leucine}$	$R_{leucine}$	R_f
alanine	0.224	0.132	0.604
arginine	0.042	0.289	0.792
asparagine	0.036	0.109	0.763
aspartic	0.000	0.078 *	0.154
cysteine	0.000	0.031	0.241
glutamic	0.006	0.184	0.347
glycine	0.091	0.281	0.416
histidine	0.097	0.113	0.763
isoleucine	0.957	0.945	0.823
leucine	1.000	1.000	0.800
phenylalanine	0.684	0.925	0.814
proline	0.303	0.414	-
serine	0.097	0.160	0.337
threonine	0.485	0.234	0.513
tryptophane	0.406	0.667	-
tyrosine	0.303 *	0.492 *	0.630
valine	0.618	0.687	0.761
lysine	0.030	0.075	0.724
methionine	0.588	0.695	0.741

* streaked

solvent 1 : n-butanol - methylethylketone - ammonia - water (5 : 3 : 1 : 1)

solvent 2 : n-butanol - glacial acetic acid - water (4 : 1 : 5)

solvent 3 : water saturated phenol

Solvent combination for second dimension of water-saturated phenol was not given, since n-butanol - glacial acetic acid solvent gave the same $R_{leucine}$ values as listed above.

Tables 53 - 67 show the relationship of the number of cells to the amount of peptide liberated by fifteen strains of psychrophiles, inoculated separately into sterilized milk. The R_f values of the peptides are given as determined by paper chromatography with three different solvent systems. Also the amino acid composition of the peptide is shown as determined by two-dimensional paper chromatography after the peptide was eluted and hydrolyzed with 6N HCl at 121°C for 8 hours. The amount of peptide liberated is expressed in terms of amino group per 100 ml of milk dialyzed against 1,500 ml of distilled water. The range 50 - 100 µl out of the final condensate of 5 ml was used for routine separation of peptides, and whenever necessary up to 1.0 ml of such condensate was used. When no spot was detected with 1.0 ml from the final condensate of 5 ml, the sample is reported as less than 1.5 µg of amino group present per 100 ml milk.

Table 53 The relationship of cell concentration of Ps. taetrolens (#1) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1*[1]	R_f 2*[2]	R_f 3*[3]	a.a. composition*[4]
0	8,800	-*[5]				
24	9,000	245.3	0.43	0.22	0.09	arginine glycine
48	12,000	-				
72	16,000	450.2	0.43	0.22	0.09	arginine glycine
96	70,000	-				
120	89,000	834.2	0.43	0.22	0.08	arginine glycine
144	150,000	-				
168	230,000	509.9	0.43	0.22	0.09	arginine glycine
192	350,000	-				

*[1] R_f 1 - R_f value determined with n-butanol - glacial acetic acid solvent

*[2] R_f 2 - R_f value determined with n-butanol - ethyl alcohol - water solvent

*[3] R_f 3 - R_f value determined with n-butanol - methylethylketone - ammonia solvent

*[4] a.a. composition - amino acid composition determined by two-dimensional paper chromatography after hydrolysis

*[5] -, not determined

Table 54 The relationship of cell concentration of Alc. viscolactis (#2) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	1,500	-				
24	2,400	83.18	0.43	0.22	-*	arginine glycine
48	3,500	-				
72	9,800	228.30	0.43	0.22	0.09	arginine glycine
96	32,500	-				
120	90,000	330.68	0.43	0.22	0.09	arginine glycine
144	175,000	-				
168	280,000	168.52	0.43	0.22	0.09	arginine glycine
192	3,000,000	-				

-*, not detectable

Symbols and abbreviation as in Table 53

Table 55 The relationship of cell concentration of Achrom. guttatus (S6) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	7,000	-				
24	12,000	<1.5	-*			
48	15,000	-				
72	17,000	39.46	0.43	0.22	-*	arginine glycine
96	77,000	-				
120	111,000	35.18	0.43	0.22	-*	arginine glycine
144	180,000	-				
168	245,000	30.49	0.43	0.22	0.10	arginine glycine
192	950,000	-				

-*, not detectable

Symbols and abbreviation as in Table 53

Table 56 The relationship of cell concentration of Ps. putrefaciens (S12) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	800	-				
24	3,800	<1.5	-*			
48	7,000	-				
72	43,000	<1.5	-*			
96	65,000	-				
120	190,000	<1.5	-*			
144	325,000	-				
168	640,000	<1.5	-*			
192	1,040,000	-				

-*, not detectable

Symbols and abbreviation as in Table 53

Table 57 The relationship of cell concentration of Ps. putrefaciens (p5) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	1,600	-				
24	2,500	75.73	0.09	0.05	0.00	histidine
48	4,200	-				
72	11,500	241.10	0.09	0.05	0.00	histidine
96	32,000	-				
120	216,000	206.90	0.09	0.05	0.00	histidine
144	235,000	-				
168	400,000	475.57	0.10	0.05	0.00	histidine
192	500,000	-				

Symbols and abbreviation as in Table 53

Table 58 The relationship of cell concentration of Alc. viscolactis (p7) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	μg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	800	-				
24	1,000	121.19	0.43	0.24	0.09	arginine glycine
48	1,100	-				
72	1,300	159.88	0.43	0.24	0.08	arginine glycine
96	4,100	-				
120	-*	134.38	0.43	0.24	0.09	arginine glycine
144	6,000	-				
168	7,000	125,85	0.425	0.24	0.09	arginine glycine
192	8,000	-				

-*, datum missing

Symbols and abbreviation as in Table 53

Table 59 The relationship of cell concentration of Ps. fragi (p8) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	68,000	<1.5	-*[1]			
24	-*[2]	-				
48	290,000	130.11	0.09	0.05	-*[1]	histidine
72	550,000	-				
96	1,000,000	87.44	0.09	0.05	-*[1]	histidine
120	2,500,000	-				
144	3,500,000	138.65	0.10	0.05	-*[1]	histidine
168	5,000,000	-				
192	15,600,000	<1.5	-*[1]			

*[1] - not detectable

*[2] - datum missing

Symbols and abbreviation as in Table 53

Table 60 The relationship of cell concentration of Alc. viscolactis (p9) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	900	<1.5	-*			
24	4,000	-				
48	13,500	357.37	0.42	0.24	0.09	arginine
						glycine
		47.99	0.09	0.05	0.00	histidine
72	46,000	-				
96	130,000	190.94	0.42	0.24	0.09	arginine
						glycine
		33.05	0.09	-*	-*	histidine ?
120	300,000	-				
144	660,000	55.24	0.42	0.24	0.09	arginine
						glycine
		<1.5	-*			
168	900,000	-				
192	1,600,000	152.54	0.42	0.24	0.09	arginine
						glycine
		28.36	0.10	0.05	-*	histidine

-*, not detectable

Symbols and abbreviation as in Table 53

Table 61 The relationship of cell concentration of Ps. fluorescens (p13) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	600	<1.5	-*			
24	3,500	-				
48	15,000	39.46	0.43	-*	-*	arginine ? glycine ?
72	50,000	-				
96	160,000	109.87	0.43	0.24	0.10	arginine glycine
120	300,000	-				
144	347,000	92.80	0.43	0.24	0.10	arginine glycine
168	550,000	-				
192	700,000	<1.5	-*			

-*, not detectable

Symbols and abbreviation as in Table 53

Table 62 The relationship of cell concentration of Ps. taetrolens ($p15^I$) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	100,000	<1.5	-*			
24	380,000	-				
48	1,100,000	117.32	0.42	0.24	0.10	arginine glycine
72	14,000,000	-				
96	20,000,000	<1.5	-*			
120	70,000,000	-				
144	110,000,000	100.24	0.09	0.05	0.00	histidine
168	250,000,000	-				
192	250,000,000	96.81	0.09	0.05	0.00	histidine

-*, not detectable

Symbols and abbreviation as in Table 53

Table 63 The relationship of cell concentration of Micro. lutens (p19) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	1,400	<1.5	-*			
24	3,600	-				
48	6,000	41.59	0.43	0.22	0.10	arginine glycine
72	8,500	-				
96	20,000	67.19	0.42	0.22	0.10	arginine glycine
120	50,000	-				
144	58,000	58.66	0.43	0.22	0.09	arginine glycine
168	200,000	-				
192	270,000	<1.5	-*			

-*, not detectable

Symbols and abbreviation as in Table 53

Table 64 The relationship of cell concentration of Alc. viscolactis (p152) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	1,400	<1.5	-*			
24	5,000	-				
48	15,000	<1.5	-*			
72	27,000	-				
96	36,000	24.52	0.42	-*	-*	arginine glycine
120	78,000	-				
144	110,000	6.10	0.43	-*	-*	arginine glycine ?
168	200,000	-				
192	320,000	<1.5	-*			

-*, not detectable

Symbols and abbreviation as in Table 53

Table 65 The relationship of cell concentration of Ps. fragi (p181) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	3,000	-				
24	14,000	126.44	0.415	0.23	0.10	arginine glycine
49	16,000	-				
72	38,000	139.74	0.42	0.225	0.09	arginine glycine
96	60,000	-				
120	100,000	43.72	0.43	0.24	0.09	arginine glycine
144	400,000	-				
168	1,450,000	<1.5	-*			
192	3,000,000	-				

-*, not detectable

Symbols and abbreviation as in Table 53

Table 66 The relationship of cell concentration of Ps. fragi (p182) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	7,000	-				
24	49,000	<1.5	-*			
48	250,000	-				
72	480,000	<1.5	-*			
96	1,800,000	-				
120	4,000,000	22.39	0.42	0.23	-*	arginine glycine
144	6,000,000	-				
168	12,000,000	<1.5	-*			
192	21,000,000	-				

-*, not detectable

Symbols and abbreviation as in Table 53

Table 67 The relationship of cell concentration of Alc. viscolactis (218) to the amount of peptide liberated from sterilized milk, and to the R_f values and amino acid composition

incubation hours	# cells	µg NH_2	R_f 1	R_f 2	R_f 3	a.a. composition
0	1,700	-				
24	3,000	80.25	0.425	0.24	0.09	arginine glycine
48	14,000	-				
72	30,000	245.32	0.43	0.24	0.10	arginine glycine
96	85,000	-				
120	100,000	152.12	0.43	0.24	0.09	arginine glycine
144	115,000	-				
168	140,000	95.61	0.43	0.24	0.09	arginine glycine
192	160,000	-				

Symbols and abbreviation as in Table 53

Table 68 and Graph 2 show the relationship between the number of cells and the amount of peptide liberated by the bacteria in 100 ml of milk, freshly drawn from the University mixed herd. The R_f values of the peptides are given as determined by paper chromatography with three different solvent systems. Also the amino acid composition of the peptide is shown as determined by two-dimensional paper chromatography after the peptide was eluted and hydrolyzed with 6 N HCl. The amount of peptides liberated is expressed in terms of amino group per 100 ml of milk dialyzed against 1,500 ml of distilled water. A range 50 - 100 µl of the final condensate of 5 ml was used for separation of peptide.

The bacteria liberated only one peptide (upon hydrolysis, arginine and glycine were identified) and a mixture of at least three amino acids, one of which was histidine.

Table 68 The relationship of cell concentration of bacteria in raw milk to the amount of peptide liberated, the R_f values and the amino acid composition

# cells	µg NH_2	R_f 1*[1]	R_f 2*[2]	R_f 3*[3]	a.a. composition
17,000	69.33	0.43	0.24	0.09	arginine glycine
487,000	120.54	0.425	0.24	0.09	arginine glycine
8,000,000	41.59	0.42	0.24	0.09	arginine glycine
14,300,000	40.21	0.42	0.24	0.09	arginine glycine
25,900,000	26.56	0.42	0.24	0.09	arginine glycine
	17.16	0.09	0.05	0.00	histidine
49,000,000	52.26	0.09	0.05	-*[4]	histidine
84,000,000	41.59	0.09	0.05	-*[4]	histidine
125,000,000	40.31	0.09	0.05	-*[4]	histidine

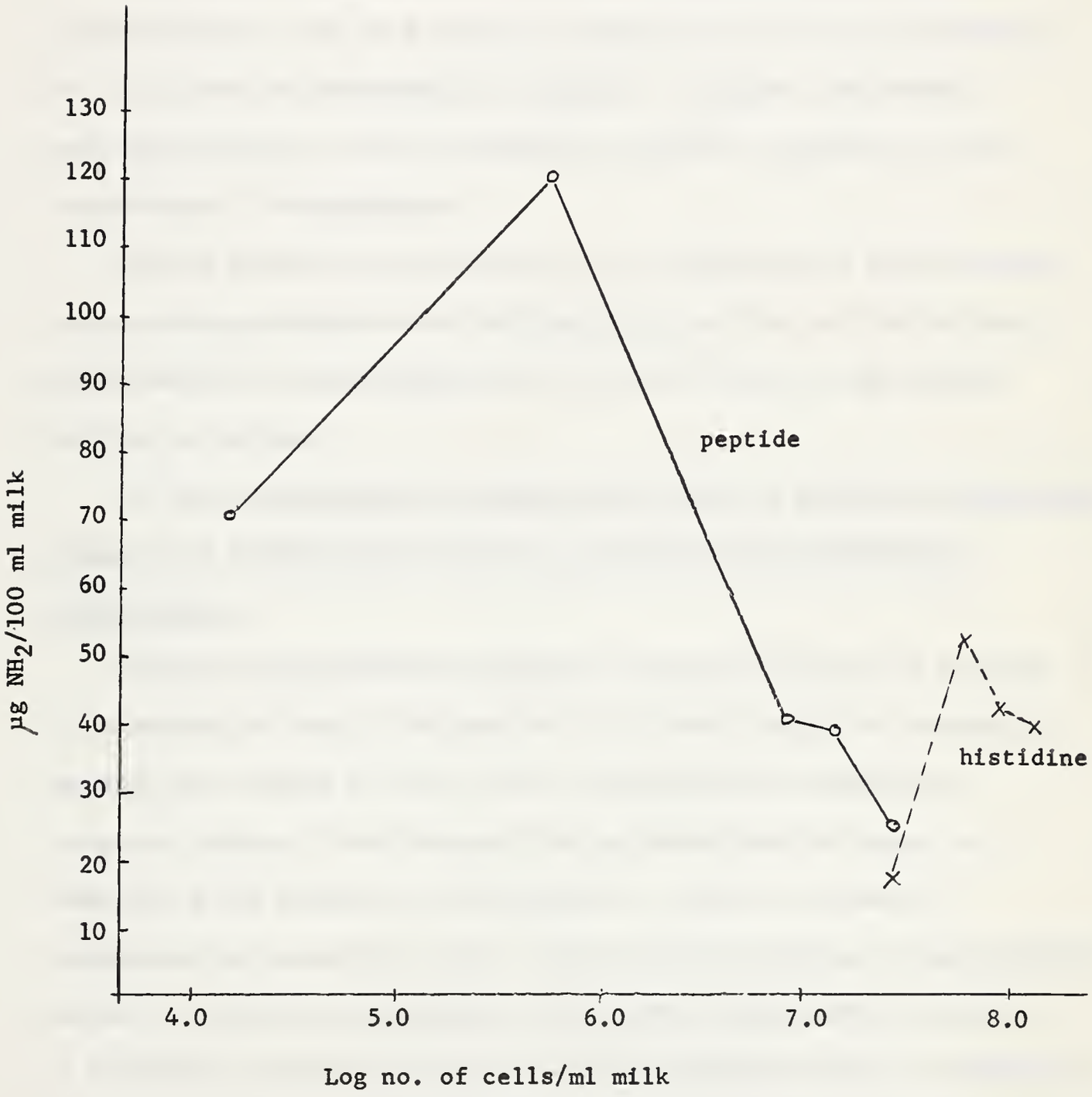
*[1] : R_f 1 - n-butanol - glacial acetic acid solvent

*[2] : R_f 2 - n-butanol - ethyl alcohol solvent

*[3] : R_f 3 - n-butanol - methylethylketone - ammonia solvent

*[4] : not detectable

Graph 2 The relationship of cell concentration of bacteria in raw milk to the amount of peptide and amino acid liberated



DISCUSSION

Identification of Psychrophilic Strains

The present studies with the fifteen strains of psychrophiles confirm the fact that an incubation temperature of 4°C is satisfactory for the growth of psychrophiles in general. However, the present work shows that 4°C is not a suitable incubation temperature for the fermentation of carbohydrates.

Several possible explanations for the difference in gas production between non-psychrophiles and psychrophiles and also for the different gas production of psychrophiles at 15° and 4°C noted in the present work are as follows.

(1) That psychrophilic organisms might carry a different carbohydrate fermentation pathway which could be a specific characteristic of psychrophiles

Lowering the incubation temperature generally lowers the kinetics of an enzyme and this is believed to be the main reason why mesophiles can only grow slowly or not at all at refrigeration temperature (Greene & Jezeski, 1954) whereas with psychrophiles the degree of lowering of the kinetics of the enzyme as a result of lowering temperature is relatively slow. Since one of the strains of psychrophiles tested in this work gave similar carbohydrate fermentation reactions at different incubation temperatures, the mechanisms which are concerned with the differences in gas production at lower incubation temperatures might not be as simple as predicted above.

(2) That the enzymes or kinetics of an enzyme are inactivated by lowering the incubation temperature

The inactivation of a particular enzyme system might activate other enzyme systems and bring about different carbohydrate fermentation pathways.

(3) That the lowering of incubation temperature might prevent cells from forming a particular enzyme

In B. stearothermophilus, for example, the lowering of the incubation temperature solidifies the lipids in the cells partially or completely, and when the incubation temperature is raised above the maximum for growth the lipids liquefy. This is believed to be one of the main causes why the bacteria cannot survive above or below a certain range of temperature. (Sobotka & Luisada - Opper, 1957). When the incubation temperature is slightly higher than that permitting maximum growth, the amount of lipid in the cell was not changed but the proportion of saturated lipids was increased.

Lowering the incubation temperature itself might not affect cell multiplication of psychrophiles but might affect the formation of a particular enzyme associated with any cell constituent which might be affected by temperature in a similar manner.

(4) Inactivation of enzymes by the combined effect of cell washing procedures and lowering the incubation temperature

The presence or absence of small amount of inducers, or the liberation of particular chemical compounds, e.g. SH group (Cohn, 1957) may have no function in cell multiplication but may participate

in gas production. The different inoculation procedures used in this work do not themselves seem to be the sole cause for the difference in gas production observed at different incubation temperatures. Further, the results obtained with non-psychrophilic pseudomonads and psychrophiles suggest that the psychrophiles have a different carbohydrate metabolic system at the lower incubation temperatures, whereas no such different system exists in non-psychrophiles.

The question now arises as to whether the lowering of the incubation temperature, perhaps in combination with different washing procedures, might have brought about a shift in the carbohydrate pathway. Some of the experimental data indicate that this is not the case. Gaughran (1949) observed that the same enzyme components for Bacillus stearothermophilus activated the respiratory system at temperatures above and below the minimum for growth. Brown et al. (1957) observed that the higher the growth temperature the more heat resistant was the pyrophosphatase of B. stearothermophilus. The optimum temperature for pyrophosphatase activity, however, did not change with the change in growth temperature.

On the other hand, Greene (1959) observed with mesophilic organism that an incubation temperature of 5°C was more readily tolerated if the organism had been previously cultured at 5°C whereas 37°C was the most favorable growth temperature when the same organism was cultivated at 37°C; this suggest adaptation of the enzyme to the 5°C incubation temperature. Similarly, Brown (1957) observed that

psychrophilic organisms were able to begin multiplication much sooner than mesophiles over almost all the temperature ranges at which both types of micro-organisms grew. The psychrophiles appeared to be better able to adjust themselves to a new environment than the mesophiles within the range 0 - 37°C.

Increasing the inoculum size generally overcame the effect of lowering the incubation temperature on rate of growth (Azuma, 1954), and shorten the generation time (Meinhart & Simmonds, 1955). However, the results obtained in the present work do not support these findings. Since the non-psychrophilic pseudomonads tested in this work did not grow as fast as the fifteen strains of psychrophiles isolated at the lower incubation temperatures, but were still able to produce gas, it is unlikely that the incubation period of 10 days, or even 15 days at 4°C was too short for the psychrophiles to produce gas. Similarly it is unlikely that the failure of the psychrophiles to produce gas at 4°C was caused by the small number of cells because several psychrophilic strains produced a greater number of cells at 4°C for 10 days than at 15°C for 3 days.

Under the incubation period used in this work, each strain had an optimum temperature for growth in a specific carbohydrate medium. In general, however, a 48 hour incubation period at 25°C was equivalent to 72 hours at 15°C and 10 days at 4°C. The growth rate at 37°C was, generally, so low that a 48 hour incubation period was not equivalent to 24 hour period at 25°C, but was more or less equivalent to a 24 hour period at 15°C. As suspected, the average

titration values at 25°C incubation exceeded the values at 15°C.

Several strains produced high titratable acidity from several different carbohydrates incubated at 25° and 15°C, but did not produce sufficient gas to be detected in the Durham's tubes, moreover there was no relationship between the degree of acidity and the amount of gas produced. Although the available information indicate that CO_2 is an end-product of glycolysis and the oxidation of pyruvic acid, the results of the present work indicate that some pseudomonads may carry different carbohydrate pathways. The strains of psychrophiles in this work might oxidize carbohydrates without decarboxylation. Weimberg (1955) observed that Ps. saccharophila carried an entirely different metabolism to break down L-arabinose from that of glucose; further he proposed a pathway whereby the oxidation did not appear to involve any phosphorylated intermediate or components in the tricarboxylic acid cycle. Pyruvic acid, according to his postulation, is not a precursor in this reaction and L-arabinose would be converted to α-ketoglutaric acid through lactone formation. Further, the metabolism of α-ketoglutarate could not be observed. A similar mechanism was postulated by Weimberg (1961) with Ps. fragi. This type of metabolism appears to be typical for hexose as well as pentose utilization in pseudomonads and also in acetobacter (Gunsalus et al., 1955).

It was intended to see if any relationship existed between the titration values and the numbers of cells or gas production. However as observed above no such relationship existed.

The results of gelatin liquefaction, especially at 37°C and 4°C

do not support the results of Pollock (1959) who postulated that if the optimum temperature for growth of the psychrophilic strains was somewhere in the neighborhood of 20°C, then the degree of gelatinase activation should have increased at this temperature. The present work indicates that the psychrophiles activated gelatinase at low temperatures providing the incubation period was prolonged, suggesting that this was brought about by an alternation in enzymatic pathway.

The results of the psychrophilic strains in litmus milk at 37°C in this work indicate that the temperature is not suitable for the growth of psychrophiles in litmus milk. Several strains failed to grow in the carbohydrate media at 37°C and with other strains the growth was not as vigorous at 37°C as at 25° or 15°C, indicating that many bacteria might not develop at 37°C sufficiently to change the reaction in litmus milk. The reaction of psychrophiles at 4°C indicates that the lowering of the incubation temperature depresses the hydrogen transport system and produces indistinct acidity, alkalinity and reduction reactions. The number of cells developed at 4°C seemed sufficient to change the reaction, since despite the indistinct reactions in the control several strains produced strong acidic and alkaline reactions. These results are in agreement with those of Kirsch et al. (1952) and Rogick & Burgwald (1952), who claimed that psychrophiles were usually biochemically inert in litmus milk when incubated at 4 - 7°C, and when the cells did produce a reaction they usually caused an increase in alkalinity. In the present work, raising the incubation temperature from 4°C to 15°C increased the acid

forming activity, but raising the temperature from 15° to 25°C and even 37°C had no further effect. This might mean that many bacteria cannot develop in litmus milk at 4°C sufficiently to change the reaction, but the degree of peptonization, where present, was increased by lowering the incubation temperature whereas coagulation and reduction occurred only at high temperatures.

If the different behavior on gas production, liquefaction of gelatin, peptonization of litmus milk, and production of α-glucosidase by strains of psychrophiles at a low incubation temperature is specific, it is possible that psychrophiles might have some specific method of utilizing proteins, lipids and carbohydrates which might be the key to indicate why a psychrophile is a psychrophile. Unfortunately this argument is supported only by limited data and the extent of knowledge on the use of the above compounds by psychrophiles is sparse. Further work is continuing on the above hypothesis but at the time of writing, insufficient data are available for inclusion here.

Ingraham & Bailey (1959) compared the glucose oxidation results of cell-free preparations of mesophiles and psychrophiles and suggested that the difference in the temperature response of psychrophiles and mesophiles was dependent on the structural integrity of the cells. On the other hand, Azuma & Clegg (unpublished data), using a Warburg apparatus at 30°C, observed that the resting cell preparations of one psychrophile when incubated at 4°C and 20°C, possibly carried different enzyme systems for each temperature.

Although for the fifteen strains of psychrophiles the results of the carbohydrate fermentation, gelatin liquefaction, litmus milk,

starch hydrolysis and nitrate reduction tests makes it defficult to classify the strains into genus and species, if differences in reaction caused by different incubation temperatures are disregarded, then they can be classified in a manner closely parallel to that of Schultze & Olson (1957).

Determination of Peptides

The psychrophiles liberated from milk a peptide which contained arginine and glycine, and occasionally an amino acid, histidine. This suggests the possibility that psychrophiles do not attack either the particular carbamyl linkage or histidine when sufficient other amino acids are available. An alternative possibility is that psychrophiles re-arrange the amino acid sequence before the liberation of excess amino acid, as postulated by Kihara et al. (1961), since the accumulation of the liberated peptide is not proportional to the cell population, and no peptide containing histidine in the amino acid composition was detected; also there may be a stage in which the liberated peptide is utilized before new protein is attacked. Kihara et al. (1961) postulated that the preferential uptake of the peptide followed by its hydrolysis would supply the limiting amino acid for the growth of Lactobacillus casei. Their results showed that although L-alanyl-D-alanine was actively absorbed by the cells, it was not hydrolyzed and hence was not used for growth, but it was accumulated intracellularly as a free peptide in a form extractable by boiling water. With the growth promoting peptide, D-alanyl-L-alanine, none of the intact peptide could be found in cell extracts; only

hydrolytic products remained.

The fact that in this work only one detectable free amino acid, histidine, was liberated can be best explained by the assumption that it was absorbed by the cells as part of the amino acid sequence in a peptide and became liberated upon intracellular hydrolysis and utilization of the remainder of the amino acids. The results of the accumulation of histidine by some strains of psychrophiles in this work is contrary to the findings of Tabor & Hayaishi (1952) and of Wickremasinghe & Fry (1954) who considered that the degradation of histidine by enzyme preparations from various micro-organisms (Pseudomonas fluorescens, Aerobacter aerogenes, Clostridium tetanomorphum) lead to the production, per mole of histidine, of 1 mole of glutamic acid, 1 mole of a C_1 compound, and 2 moles of ammonia. Those strains of psychrophiles which accumulated histidine seems to be deficient in histidine-α-deaminase.

Kihara & Snell (1960) suggested that with L. casei, a growth factor present in partial hydrolysates of proteins "strepogenin" stimulated early growth in a defined medium, and a common cause of this proved to be an inbalance among the amino acids of the medium so that an excess of one amino acid inhibited the utilization for growth of a structurally related amino acid but not that of its peptide. The peptides, however, apparently served only to supply a limiting amino acid, the free form of which was not utilized efficiently for growth, but rather to supply a unique sequence of amino acids. The situation in the present experiments, where the liberation of peptides

started rather late in the stage of incubation, is different to that of Kihara & Snell where the peptides were present initially. However, there is no precise explanation why the liberated peptide is utilized before new protein is attacked.

Oginsky & Gehrig (1952) observed that Streptococcus faecalis, as well as Neurospora crassa, contained an enzyme, arginine deaminase, which converts arginine to citrulline and ammonia. It is uncertain whether the gradual disappearance of the peptide in this work can be fully explained by the slow production by the psychrophilic organisms of the enzyme or by the slow utilization of amino acids, arginine and glycine, since the extent of knowledge of the enzyme system in the different genera is sparse.

The carbon atoms of glycine are utilized for the synthesis of a number of tissue constituents, and in some strictly anaerobic bacteria this is converted to acetic acid. Nisman (1954) postulated that Cl. sporogenes and Cl. botulinum used the "Stickland reaction" to utilize glycine, whereby some amino acids act as reducing agents (e.g. alanine) and others that act as oxidizing agents (e.g. glycine). In one instance with an E. coli mutant which grew either on L-serine or glycine, Meinhart & Simmonds (1955) observed that glycine dipeptides, other than glycylglycine, were much more efficient than an equimolar amount of free amino acid in supporting the growth of the organism; furthermore the presence of alanine interfered with the uptake of the peptides by the organism.

It is possible that in the present work, before such reducing agents are utilized efficiently, the dipeptide containing arginine

and glycine remains intact and is accumulated; further by the uptake of reducing agents, the dipeptide enhanced the growth of the cells and is absorbed by them.

Although there is a relationship between the sudden increase in number of cells and the decrease in the amount of peptide liberated, more experimental data are necessary before it can be concluded that the accumulated peptide stimulated the cell multiplication. A hypothesis proposed by Witter & Tuckey (1960) that accumulated intermediates may have stimulated the cell multiplication resulting in a sudden shortening of the generation time cannot be rejected. However, in this work such a shortening of generation time occurred at rather an early state of incubation. The generation rates for the first 48 hours showed quite remarkable differences with different strains. The fact that in these experiments the results were different to the findings of Witter & Tuckey may be explained by differences in the activity of the strains used.

The results from the liberated peptide cast doubt on the existence of a carbamyl linkage in natural milk proteins, since there is no sound knowledge on either the nature of proteinases (Cohen & Gros, 1960) or the type of proteinases produced by bacteria (Brandsaeter & Nelson, 1956a, b). The results of the degree of peptonization in litmus milk did not show a close relationship with the amount of peptide and free amino acid liberated by the psychrophiles, suggesting that different species and even different strains within the same species may attack milk proteins differently, as observed by Camp & Van der Zant (1957)

with a mesophile.

Although the results of individual trials with the isolated psychrophiles showed a variable relationship between the number of cells and the amount of peptide liberated, the trial with raw milk samples gave a fairly reasonable relationship. The results might be more clearly understood by the calcellation of the two extreme examples, which in one case utilized protein so rapidly that no appreciable amount of peptide was liberated, and the another in which the protein was utilized slowly and peptide was accumulated. The applicability of this method to predict the number of cells present in milk, and its keeping quality, is limited since the type, number and the activity of bacteria in milk influence the amount of peptide liberated and utilized.

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